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FEE TRANSMITTAL

Attorney Docket No.	22338-01207
Application Number	09/993,234
Filing Date	November 19, 2001
First Named Inventor	Avi J. ASHKENAZI
Group Art Unit	1642
Examiner Name	Gary B. Nickol

AMOUNT ENCLOSED	0.00

FEE CALCULATION (fees effective 12/08/03)

CLAIMS AS AMENDED	Claims Remaining After Amendment	Highest Number Previously Paid For	Number Extra	Rate	Calculations
TOTAL CLAIMS	5	- 20 =	0	X $50.00 =	$ 0.00
INDEPENDENT CLAIMS	1	- 3 =	0	X $200.00 =	0.00

Since an Official Action set an original due date of August 14, 2005, petition is hereby made for an extension to cover the date this reply is filed for which the requisite fee is enclosed (1 month ($120)); (2 months ($450)); (3 months ($1,020)); (4 months ($1,590)); (5 months ($2,160):	2160.00
Appeal Brief, add fee ($500.00)	500.00
If Statutory Disclaimer under Rule 20(d) is enclosed, add fee ($130.00)	
Information Disclosure Statement (Rule 1.17(p)) ($180.00)	
Total of above Calculations =	$ 2660.00
Reduction by 50% for filing by small entity (37 CFR 1.9, 1.27 & 1.28)	$0.00
TOTAL FEES DUE =	$ $2660.00

(1) If entry (1) is less than entry (2), entry (3) is "0".
(2) If entry (2) is less than 20, change entry (2) to "20".
(4) If entry (4) is less than entry (5), entry (6) is "0".
(5) If entry (5) is less than 3, change entry (5) to "3".

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Deposit Account Name	**Sidley Austin LLP**

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SUBMITTED BY:

Typed Name	David A. Steffes	Reg. No.	46,042
Signature	David A. Steffes	Date	1/17/06

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application No.:	09/993,234	Confirmation No.:	1337
Applicant:	Avi J. ASHKENAZI		
Filed:	November 19, 2001		
Group Art Unit:	1642		
Examiner:	Gary B. Nickol		
Docket No.:	22338-01207		
Customer No.:	33,694		

APPEAL BRIEF

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Washington, D.C. 20231

Dear Sir:

Appellant files this Appeal Brief pursuant to the provisions of 37 C.F.R. § 1.192 from the rejection in the Office Action mailed 14 December 2004. Since a Notice of Appeal was filed on 14 June 2005 in connection with the above-identified application, an Appeal Brief was due 14 August 2005. A Petition for an Extension of Time of five (5) months accompanies this Appeal Brief. January 14, 2006 fell on a Saturday, and Monday, January 16, 2006 was a federal holiday. Accordingly, this Appeal Brief is considered timely filed on Tuesday, January 17, 2006.

TABLE OF CONTENTS

Herewith, Appellant submits (a) an original and two copies of this Appeal Brief; (b) an Appendix of Claims on Appeal, attached hereto as Exhibit A; and (c) the following additional Exhibits:

- Exhibit B: Specification and Claims of U.S. Provisional Application No. 60/013,285
- Exhibit C: Specification and Claims of U.S. Provisional Application No. 60/028,711

1. REAL PARTY IN INTEREST

The real party in interest in this appeal is Genentech, Inc. by virtue of an assignment recorded in the U.S. Patent and Trademark Office on September 2, 1997 at Reel/Frame: 008690/0298, in connection with parent U.S. Application No. 08/828,683, now U.S. Patent No. 6,469,144.

2. RELATED APPEALS AND INTERFERENCES

There are no other Appeals or Interferences known to the appellants, the appellants' legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the present pending appeal.

3. STATUS OF CLAIMS

Claims 1-45 were originally filed. Claims 46-94 were added and original claims 1-33 and 40-45 were cancelled at the time of filing in a preliminary amendment. Claims 35 and 46-94 were withdrawn by virtue of a Restriction Requirement maintained by the Examiner. Claims 34 and 36-39 were rejected in an Office Action dated October 7, 2003. Claims 34 and 36-39 were finally rejected in an Office Action dated December 14, 2004. The claims involved in this appeal, claims 34 and 36-39, are presented in the appendix attached hereto as Exhibit A.

4. STATUS OF AMENDMENTS

There are no outstanding amendments.

5. SUMMARY OF THE INVENTION

In one aspect, the present invention relates to an isolated nucleic acid encoding Apo-3 polypeptide comprising amino acid residues 1 to 417, 25 to 417, 25 to 198, or 338 to 417 of SEQ ID NO: 6, or a biologically active variant thereof. In another aspect, the present invention also relates to a vectors and host cells comprising the isolated nucleic acid.

6. ISSUES

A. Whether descriptive support is provided for claim 34 under 35 U.S.C. § 112, first paragraph.

B. Whether claims 34 and 36-39 are anticipated, under 35 U.S.C. § 102(e), by U.S. Patent No. 6,153,402 ("Yu *et al.*").

7. GROUPING OF CLAIMS

Appellants hereby state that the pending claims should be considered as a single group. Patentability of this group of claims resides, at least in part, in the recitation of the amino acid sequence of SEQ ID NO: 6 and specific regions thereof.

8. ARGUMENT

A. Claim 34 is supported by the disclosure in compliance with 35 U.S.C. § 112, first paragraph

Pending claim 34 was rejected under 35 U.S.C. § 112, first paragraph, as not supported by the specification. The Examiner has stated that "an isolated nucleic acid encoding Apo-3

polypeptide comprising amino acid residues 25-417 has no clear support in the specification and the claims as originally filed." December 14, 2004 Office Action, page 4.

Amino acid residues 1-24 of SEQ ID NO:6 comprise a signal sequence. *See, e.g.*, the specification at page 20, lines 8-9; Figure 4. As described in the specification, amino acid residues 25-417 of SEQ ID NO:6 comprise the extracellular domain, transmembrane domain and intracellular domain of the Apo-3 polypeptide. *See, e.g.*, the specification at page 20, lines 9-11. Secreted forms of the Apo-3 polypeptide are contemplated and encompassed by the present claims. *See, e.g.*, the specification at page 13, lines 20-24. Cleavage of the Apo-3 signal sequence during secretion of the soluble protein yields a polypeptide having amino acid residues 25-417 of SEQ ID NO:6. Moreover, the specification at page 23, line 6-31, discusses certain aspects of the signal sequence component. Signal sequences incorporating a specific cleavage site at the N-terminus of the mature protein, which are subsequently cleaved (yielding a polypeptide having amino acid residues 25-417 of SEQ ID NO:6) are described in this section. *See, e.g.*, the specification at page 23, lines 13-15. In any event, the present disclosure contemplates biologically active Apo-3 polypeptides having the amino acid sequence of SEQ ID NO:6, wherein "from about one to 24 amino acid residues are deleted." *See, e.g.*, specification page 14, lines 1-4. This would include a polypeptide having amino acid residues 25-417 of SEQ ID NO:6.

Accordingly, an isolated nucleic acid encoding Apo-3 polypeptide comprising amino acid residues 25-417 of SEQ ID NO:6 is adequately described in the present specification.

B. Claims 34 and 36-39 are not anticipated by Yu *et al.* under 35 U.S.C. § 102(e)

Pending claims 34 and 36-39 were rejected under 35 U.S.C. § 102(e) as anticipated by Yu *et al.* The Examiner has stated that "applicants have not provided any clear evidence that the disclosure of DR3 or DR3-V1 does not anticipate the currently claimed subject matter." In addition, the Examiner has stated that "Yu *et al.* clearly taught an isolated nucleic acid encoding

amino acid residues 25-198 . . . [set forth] on page 67 of US Provisional 60/013,285 beginning at amino acid position No. 36."

It has been the Appellant's position that Yu *et al.* is not an appropriate reference under 35 U.S.C. § 102(e) because the priority application relied on for this citation, U.S. Provisional Application No. 60/013,285, fails to teach all of the elements, and indeed the isolated nucleic acid encoding Apo-3 polypeptide, of pending claim 34.

For purposes of the present argument, the Appellant notes that the Examiner has accorded the pending claims a priority date of September 23, 1996.[1] Yu *et al.* claims priority through three different provisional applications — U.S. Provisional Application No. 60/037,341, filed February 6, 1997; U.S. Provisional Application No. 60/028,711, filed October 17, 1996; and U.S. Provisional Application No. 60/013,285, filed March 12, 1996 ("the '285 application"). Only the earliest of these provisional applications, the '285 application, pre-dates the priority date currently accorded the pending claims. Therefore, the only relevant disclosure for determining whether Yu *et al.* anticipates under 35 U.S.C. § 102(e) is that of the '285 application. *See, e.g., In re Wertheim and Mishkin*, 209 USPQ 554 (CCPA 1981).

Yu *et al.* discloses two different polypeptides, referred to as DR3-V1 and DR3 respectively, encoded by cDNA nucleic acid sequences which were cloned from a cDNA library. The '285 application, however, disclosed *only* the sequence of DR3-V1[2]; the second sequence, DR3, was disclosed *for the first time* in the second priority application of Yu *et al.* filed October 17, 1996 and cannot, therefore, be prior art to the instant application. The fact that only DR3-V1 was disclosed in the earliest provisional application is significant because the DR3-V1 polypeptide, as disclosed in the '285 application, does not correspond to the Apo-3 polypeptide

[1] In order to minimize the issues on appeal Appellant accepts this priority date only for purposes of the present argument. The earliest claimed priority date of the present application is April 1, 1996. The Appellant expressly reserves the right to dispute the priority accorded to the present claims by the Examiner and to establish earlier priority than accorded the present claims.
[2] The '285 application refers to DR3-V1 as "DDCR." *See* Yu *et al.* Col. 3, ln. 22.

in overall sequence or the particular regions identified in the present claims. This is clearly evident by the alignment of the signal peptides of the DR3-V1 sequence disclosed in the '285 application and Apo-3 polypeptides below (bolded amino acid residues indicate identical residues).

```
DR3-V1:   1   Met Glu Glu Thr Gln Gln Gly Glu Ala Pro 10
Apo-3:    1   Met Glu Gln Arg Pro Arg Gly Cys Ala Ala 10

DR3-V1:   11  Arg Gly Gln Leu Arg Gly Glu Ser Ala Ala 20
Apo-3:    11  Val Ala Ala Ala Leu Leu Leu Val Leu Leu 20

DR3-V1:   21  Pro Val Pro Gln Ala Leu Leu Leu Val Leu 30
Apo-3:    21  Gly Ala Arg Ala 24
```

As is clearly evident, the signal peptides of DR3-V1 and Apo-3 are very different when aligned from the first amino acid residue of each polypeptide. The Appellant notes that there is no indication in the '285 application that the deduced DR3-V1 polypeptide should be compared with other proteins, if at all, in any other way than from the first amino acid residue.

An alignment of the designated extracellular domains of the DR3-V1 and Apo-3 polypeptides is as follows:[3]

```
DR3-V1:   30  Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr 39
Apo-3:    25  Gly Gly Thr Arg Ser Pro Arg Cys Asp Cys 34

DR3-V1:   40  Arg Ser Pro Arg Cys Asp Cys Ala Gly Asp 49
Apo-3:    35  Ala Gly Asp Phe His Lys Lys Ile Gly Leu 44

DR3-V1:   50  Phe His Lys Lys Ile Gly Leu Phe Cys Cys 59
Apo-3:    45  Phe Cys Cys Arg Gly Cys Pro Ala Gly His 54

DR3-V1:   60  Arg Gly Cys Pro Ala Gly His Tyr Leu Lys 69
Apo-3:    55  Tyr Leu Lys Ala Pro Cys Thr Glu Pro Cys 64

DR3-V1:   70  Ala Pro Cys Thr Glu Pro Cys Gly Asn Ser 79
Apo-3:    65  Gly Asn Ser Thr Cys Leu Val Cys Pro Gln 74
```

[3] This region is referred to as the "ligand binding domain" in the '285 application.

DR3-V1:	80	Thr	Cys	Leu	Val	Cys	Pro	Gln	Asp	Thr	Phe	89
Apo-3:	75	Asp	Thr	Phe	Leu	Ala	Trp	Glu	Asn	His	His	84
DR3-V1:	90	Leu	Ala	Trp	Glu	Asn	His	His	Asn	Ser	Glu	99
Apo-3:	85	Asn	Ser	Glu	Cys	Ala	Arg	Cys	Gln	Ala	Cys	94
DR3-V1:	100	Cys	Ala	Arg	Cys	Gln	Ala	Cys	Asp	Glu	Gln	109
Apo-3:	95	Asp	Glu	Gln	Ala	Ser	Gln	Val	Ala	Leu	Glu	104
DR3-V1:	110	Ala	Ser	Gln	Val	Ala	Leu	Glu	Asn	Cys	Ser	119
Apo-3:	105	Asn	Cys	Ser	Ala	Val	Ala	Asp	Thr	Arg	Cys	114
DR3-V1:	120	Ala	Val	Ala	Asp	Thr	Arg	Cys	Gly	Cys	Lys	129
Apo-3:	115	Gly	Cys	Lys	Pro	Gly	Trp	Phe	Val	Glu	Cys	124
DR3-V1:	130	Pro	Gly	Trp	Phe	Val	Glu	Cys	Gln	Val	Ser	139
Apo-3:	125	Gln	Val	Ser	Gln	Cys	Val	Ser	Ser	Ser	Pro	134
DR3-V1:	140	Gln	Cys	Val	Ser	Ser	Ser	Pro	Phe	Tyr	Cys	149
Apo-3:	135	Phe	Tyr	Cys	Gln	Pro	Cys	Leu	Asp	Cys	Gly	144
DR3-V1:	150	Gln	Pro	Cys	Leu	Asp	Cys	Gly	Ala	**Leu**	His	159
Apo-3:	145	Ala	Leu	His	Arg	His	Thr	Arg	Leu	**Leu**	Cys	154
DR3-V1:	160	Arg	His	Thr	Arg	Leu	Leu	**Cys**	Ser	Arg	Arg	169
Apo-3:	155	Ser	Arg	Arg	Asp	Thr	Asp	**Cys**	Gly	Thr	Cys	164
DR3-V1:	170	Asp	Thr	Asp	Cys	Gly	Thr	Cys	Leu	Pro	**Gly**	179
Apo-3:	165	Leu	Pro	Gly	Phe	Tyr	Glu	His	Gly	Asp	**Gly**	174
DR3-V1:	180	Phe	Tyr	Glu	His	Gly	Asp	Gly	Cys	Val	Ser	189
Apo-3:	175	Cys	Val	Ser	Cys	Pro	Thr	Ser	Thr	Leu	Gly	184
DR3-V1:	190	Cys	Pro	Thr	Ser	Thr	Leu	Gly	Ser	Cys	Pro	199
Apo-3:	185	Ser	Cys	Pro	Glu	Arg	Cys	Ala	Ala	Val	Cys	194
DR3-V1:	200	Glu	Arg	Cys	Ala	Ala	Val	Cys	Gly	Trp	Arg	209
Apo-3:	195	Gly	Trp	Arg	Gln	198						
DR3-V1:	210	Gln	Met	Phe	Trp	Val	215					

As is also evident from the above alignment, the extracellular domains of DR3-V1 and Apo-3 are very different.

Finally, an alignment of the designated death domains of the DR3-V1 and Apo-3 polypeptides is as follows:

```
DR3-V1:   350  Met Asp Ala Val Pro Ala Arg Arg Trp Lys 359
Apo-3:    338  Val Met Asp Ala Val Pro Ala Arg Arg Trp 347

DR3-V1:   360  Glu Phe Val Arg Thr Leu Gly Leu Arg Glu 369
Apo-3:    348  Lys Glu Phe Val Arg Thr Leu Gly Leu Arg 357

DR3-V1:   370  Ala Glu Ile Glu Ala Val Glu Val Glu Ile 379
Apo-3:    358  Glu Ala Glu Ile Glu Ala Val Glu Val Glu 367

DR3-V1:   380  Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met 389
Apo-3:    368  Ile Gly Arg Phe Arg Asp Gln Gln Tyr Glu 377

DR3-V1:   390  Leu Lys Arg Trp Arg Gln Gln Gln Pro Ala 399
Apo-3:    378  Met Leu Lys Arg Trp Arg Gln Gln Gln Pro 387

DR3-V1:   400  Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu 409
Apo-3:    388  Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu 397

DR3-V1:   410  Arg Met Gly Leu Asp Gly Cys Val Glu Asp 419
Apo-3:    398  Glu Arg Met Gly Leu Asp Gly Cys Val Glu 407

DR3-V1:   420  Leu
Apo-3:    408  Asp Leu Arg Ser Arg Leu Gln Arg Gly Pro 417
```

As is similarly evident from the above alignment, the death domains of DR3-V1 and Apo-3 are markedly different.

To the extent the '285 application contemplates fragments of the deduced DR3-V1 polypeptide, no fragments having any likeness to the Apo-3 polypeptide of the present claims are identified in the '285 application. Moreover, the '285 application fails to discuss any specific sequence frame shifts, inversions, repeats, additions or deletions that would result in a polypeptide having any likeness to the Apo-3 polypeptide of the present claims. It is possible that the Examiner may have relied on such alterations in the deduced DR3-V1 polypeptide

sequence but has not identified where in the '285 application one may locate the basis for the suggested alterations nor indicated that they are inherent aspects of the deduced DR3-V1 polypeptide. For example, although the Examiner has noted a comparison of a segment of the deduced DR3-V1 polypeptide starting at amino acid position No. 36 with a portion of the Apo-3 polypeptide of the present claims, this alignment appears to be impermissibly based either on the knowledge of the sequence of the present claims or the disclosure from Yu *et al.* (*see, e.g.*, Col. 5, line 63) that was not included in the '285 application. Such an alignment of the sequences beginning at amino acid position No. 36 does not involve any region of interest identified in the '285 application. The '285 application provides no indication that a region of the deduced DR3-V1 polypeptide starting at amino acid position No. 36 exists as a separate polypeptide, begins a region of interest in the DR3-V1 polypeptide (*i.e.*, extracellular domain), or has any significance whatsoever.

Accordingly, the above alignments provide evidence supporting the Appellant's position that the deduced DR3-V1 polypeptide does not correspond to, and therefore does not anticipate under § 102(e), the Apo-3 polypeptide of the present claims.

In addition, to the extent the '285 application provides any direction to a signal peptide (amino acid residues 1-30 – *see* page 7, lines 34-35), extracellular domain (amino acid residues 30-215 - *see* page 7, line 35), transmembrane domain (amino acid residues 215-240 – *see* page 8, line 1), intracellular domain (amino acid residues 240-428 – *see* page 8, lines 1-2) or death domain (amino acid residues 350-420 – *see* page 8, lines 2-3), it points to completely different domains having different boundaries within its longer 428 amino acid DR3-V1 (DDCR) polypeptide versus the polypeptide of the present claims.[4] *See* '285 application at page 7, line

[4] *See In re Arkley*, 172 USPQ 524 (CCPA 1972) ("[F]or the instant rejection under 35 USC 102(e) to have been proper, the . . . reference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without any need for picking, choosing, and combining various disclosures not directly related to each other by the teachings of the cited reference."); *see also Suntiger, Inc. v. Blublocker*, 51 USPQ2d 1811, 1818 (Fed. Cir. 1999) ("The case law makes clear that disclosure of a generic expression

34-page 8, line 3; page 11, lines 10-19; Figure 1; SEQ ID NO:1 as is illustrated in the sequence alignments above.

In this regard, the Appellant notes that the domains (*e.g.*, the signal sequence, extracellular domain, transmembrane domain, intracellular domain and death domain) of DR3-V1 set forth in Yu *et al.* are *not* those set forth for DR3-V1 in the '285 application. Compare the '285 application at page 7, line 34-page 8, line 3; page 11, lines 10-19 with Yu *et al.* at col. 4, lines 25-31. The table below summarizes the changes to these domains between the '285 application and Yu *et al.*:

DR3-V1 Domain	**'285 application (SEQ ID NO:2) Amino Acid Position Nos.**	**Yu *et al.* (SEQ ID NO:2) Amino Acid Position Nos.**
Signal peptide	1-30	1-35
Extracellular domain	30-215	36-212
Transmembrane domain	215-240	213-235
Intracellular domain	240-428	236-428
Death domain	350-420	353-419

Each of these domains were changed, without explanation, in applications filed after the '285 application. *See id.*; *see also* Application No. 60/028,711 at page 6, lines 15-19. One likely explanation for these changes is that sometime between the filing of the '285 and the application giving rise to Yu *et al.*, the inventors of the '285 application realized that they inaccurately estimated the positions of each of the DR3-V1 polypeptide domains in the '285 application. In fact, Yu *et al.* acknowledges that the position of the signal sequence cleavage site, for example, may have been predicted using a method having only about 75-80% accuracy. *See* Yu *et al.* at

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encompassing a large number of possible variants is not a description of all of them." *Id.* (citing In re *Ruschig*, 154 USPQ 118, 121 (CCPA 1967) (finding that a disclosure of a class of compounds did not provide descriptive support for a single compound within the class *not specifically identified* in the specification); and *Fujikawa v. Wattanasin*, 39 USPQ2d 1895, 1904-05 (Fed. Cir. 1996) (finding no descriptive support even when the proposed genus was actually disclosed in the specification due to the *lack of direction* that would lead one of skill to the proposed subgenus)).

col. 6, lines 50-59 (a disclosure that was provided in Yu *et al.*, but not in the '285 application). Clearly, a question of descriptive support for, and enablement of, any DR3-V1 polypeptide that may exist in nature arises in light of the comparison of the '285 application and Yu *et al.* disclosures. Moreover, confusion by the Yu *et al.* and '285 application inventors about aspects of the deduced DR3-V1 polypeptide cannot be reasonably interpreted as an inherent disclosure of the Apo-3 polypeptide of the present claims, with each of its limitations. *See Continental Can Co. USA, Inc. v. Monsanto Co.*, 948 F.2d 1264, 1269, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) ("Inherency, however, may not be established by probabilities or possibilities.").

Accordingly, the Yu *et al.* reference is not an effective anticipatory reference under 35 U.S.C. § 102(e) as its priority applications do not describe, in a manner sufficient under § 112, each of the limitations of the pending claims.

9. CONCLUSION

Appellants have addressed each of the rejections set forth by the Examiner. For the reasons stated above, it is respectfully submitted that the final rejections of claims 34 and 36-39 under 35 U.S.C. § 112, first paragraph, and § 102(e) are in error and warrant reversal of the rejections by the Board.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, appellant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 18-1260** referencing docket no. 2233801207. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

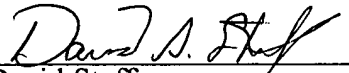
Dated: January 17, 2006 By: 

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Exhibit B

96941942-3 PCT/US 96/16849

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BAR CODE LABEL

U.S. PATENT APPLICATION

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT
60/013,285 PROVISIONAL	03/12/96		

APPLICANT

GUO-LIANG YU, DARNESTOWN, MD; JIAN HI, ROCKVILLE, MD; REINER L. GENTZ, SILVER SPRING, MD.

CONTINUING DATA********
VERIFIED

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FOREIGN/PCT APPLICATIONS********
VERIFIED

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PRIORITY DOCUMENT

FOREIGN FILING LICENSE GRANTED 04/12/96

STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
MD	4			$150.00	PF267PP

ADDRESS

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TITLE

DEATH DOMAIN CONTAINING RECEPTOR

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Date NOV 5 1996 Certifying Officer

PATENT APPLICATION SERIAL NO. 60/013285

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

P507069 03/27/96 60013285 08-3425 070 114 150.00CH PF267PP

PTO-1556
(5/87)

DEATH DOMAIN CONTAINING RECEPTOR

The present invention relates to a novel member of the tumor necrosis factor family of receptors.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

For example, tumor necrosis factors (TNF)alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and nerve growth factor (NGF). The superfamily of TNF

receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, X40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295 (1994).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature, 356:314 (1992), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C., et al., Science, 259:990 (1993). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F., et al., Cell, 69:737 (1992).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von

Huffel, C., Science, 264:667, 668 (1994). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia *et al.*, *Cell* 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

SUMMARY OF THE INVENTION

The present inventors have discovered a novel member of the tumor necrosis factor (TNF) family of receptors. By the invention, isolated nucleic acid molecules are provided comprising nucleic acid sequences encoding a death domain containing receptor (DDCR), and fragments thereof. The DDCR gene contains an open reading frame encoding a protein of about 428 amino acid residues whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO. 1], with a leader sequence of about 30 amino acid residues, and a deduced molecular weight of about 47 kDa. Of known members of the TNF receptor family, the DDCR polypeptide shares the greatest degree of homology with human TNF R1.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] and

further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] but which, due to the degeneracy of the genetic code, still encode the DDCR polypeptide or a fragment thereof.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DDCR ligand binding domain; a polypeptide comprising the DDCR transmembrane domain; a polypeptide comprising the DDCR intracellular domain; and a polypeptide comprising the DDCR death domain.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode for fragments, analogs or derivatives of the DCCR polypeptide. Variants may occur naturally, such as an allelic variant. Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Further embodiments of the invention include isolated nucleic acid molecules that are at least 70% identical, and more preferably at least 80%, 90%, 95%, 97%, 98% or 99% identical, to the nucleic acid sequence shown in Figure 1 [SEQ ID NO 1], to fragments complementary thereto, or to fragments thereof selected from (1) a fragment that encodes the mature DDCR protein (i.e., the open reading frame), (2) a fragment that encodes the DDCR ligand binding domain, (3) a fragment that encodes the DDCR transmembrane domain, (4) a fragment that encodes the DDCR intracellular domain, and (5) a fragment that encodes the death domain.

The invention is also related to the use of the DDCR polynucleotides for detecting altered expression of DDCR transcripts, such as, for example, during apoptosis dysfunction associated with tumors or autoimmune disease.

The isolated nucleic acid molecules of the present invention are also valuable for chromosome identification.

The DDCR nucleotide sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome.

The present invention also provides vectors and host cells for recombinant expression of the nucleic acid molecules described herein.

Further provided are isolated DDCR polypeptides having the amino acid sequence shown in Figure 1 [SEQ ID NO 2], or fragments thereof. The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the DDCR polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 70% similarity (preferably at least 70% identity) to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

The invention further provides DDCR polypeptide fragments selected from the mature DDCR protein, the DDCR ligand binding domain, the DDCR transmembrane domain, the DDCR intracellular domain, and the DDCR death domain. Such fragments of DDCR can be used to raise polyclonal and monoclonal antibody-agonists and antagonists capable of enhancing or inhibiting the DDCR ligand/receptor signaling pathway. Further, such fragments can be used in the yeast two-hybrid system to "capture" DDCR binding proteins which are

also candidate agonist and antagonist according to the present invention.

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of DDCR protein, or the soluble form thereof, in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DDCR, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

Tumor Necrosis Factor(TNF)family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. Cellular response to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

Thus, the invention further provides a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DDCR polypeptide an effective amount of an agonist capable of increasing DDCR mediated signaling. Preferably, DDCR mediated signaling is increased to treat a disease wherein

decreased apoptosis is exhibited.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DDCR polypeptide an effective amount of an antagonist capable of decreasing DDCR mediated signaling. Preferably, DDCR mediated signaling is decreased to treat a disease wherein increased apoptosis is exhibited.

Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below. Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DDCR polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By the invention, a cell expressing the DDCR polypeptide can be contacted with either an endogenous or exogenously administed TNF-family ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide and deduced amino acid sequence of DDCR. Amino acids 1 to 30 constitute the signal peptide, amino acids 30-215 the ligand binding domain, amino

acids 215-240 the transmembrane domain, amino acids 240-428 the intracellular domain, and amino acids 350-420 the death domain.

Figure 2 shows the regions of similarity between the amino acid sequences of the DDCR (referred to in the Figure as HTTNB61.pep), human tumor necrosis factor receptor 1, and Fas receptor (SEQ ID NOs:3 and 4).

Figure 3 shows structural and functional features of DDCR deduced by the indicated techniques, as a function of amino acid sequence.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a nucleic acid sequence encoding the DDCR polypeptide whose amino acid sequence is shown in Figure 1 [SEQ ID NO:2] or a fragment of the polypeptide. The DDCR polypeptide of the present invention shares sequence homology with human TNF RI and Fas (Figure 3). The nucleotide sequence shown in Figure 1 [SEQ ID NO:1] was obtained by sequencing the HTTNB61 clone, which was deposited on February 29, 1996 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given accession number 97456.

Thus, in one aspect, isolated nucleic acid molecules are provided which encode the DDCR polypeptide. By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vitro* RNA transcripts of the DNA

molecules of the present invention. By "isolated" polypeptide or protein is intended a polypeptide or protein removed from its native environment. For example, recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention as are native or recombinant polypeptides which have been substantially purified by any suitable technique such as, for example, the single-step purification method disclosed in Smith and Johnson, *Gene* 67:31-40 (1988).

Using the information provided herein, such as the nucleic acid sequence set out in Figure 1, a nucleic acid molecule of the present invention encoding a DDCR polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figure 1 was discovered in a cDNA library derived from cells of a human testis tumor.

The DDCR gene contains an open reading frame encoding a protein of about 428 amino acid residues whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO. 1], with a leader sequence of about 30 amino acid residues, and a deduced molecular weight of about 47 kDa. Of known members of the TNF receptor family, the DDCR polypeptide of the invention shares the greatest degree of homology with human TNF R1. (The DDCR polypeptide shown in Figure 1 [SEQ ID NO 2] is about 20% identical and about 50% similar to human TNF R1.)

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 198-200 of the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] but which, due to the degeneracy of the genetic code, still encode the DDCR polypeptide or a fragment thereof. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above.

The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 [SEQ ID NO 1], or a fragment thereof. Such isolated DNA molecules and fragments thereof are useful as DNA probes for gene mapping by *in situ* hybridization with chromosomes and for detecting expression of the DDCR gene in human tissue (including testis tumor tissue) by Northern blot analysis. Of course, as discussed above, if a DNA molecule includes the ORF whose initiation codon is at position 198-200 of Figure 1 [SEQ ID NO 1], then it is also useful for expressing the DDCR polypeptide or a fragment thereof.

By fragments of an isolated DNA molecule having the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] are intended DNA fragments at least 20 bp, and more preferably at least 30 bp in length which are useful as DNA probes as discussed above. Of course larger DNA fragments 50-1500 bp in length are also useful as DNA probes according to the present invention as are DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in Figure 1 [SEQ ID NO 1]. By a fragment at least 20 bp in length, for example, is intended fragments which include 20 or more bases from the nucleotide sequence in Figure 1 [SEQ ID NO 1].

Since the nucleotide sequence shown in Figure 1 [SEQ ID NO 1] is provided, generating such DNA fragments would be routine to the skilled artisan. For example, restriction endonuclease cleavage or shearing by sonication could easily be used to generate fragments of various sizes. Alternatively, the DNA fragments of the present invention could be generated synthetically according to known techniques.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DDCR ligand binding domain (amino acid residues from about 30 to about 215 in Figure 1 [SEQ ID NO 2]); a polypeptide comprising the DDCR transmembrane domain (amino acid residues from about 215 to about 240 in Figure 1 [SEQ ID NO 2]; a polypeptide comprising the DDCR intracellular domain (amino acid residues from about 240 to about 428 in Figure 1 [SEQ ID NO 2]; and a polypeptide comprising the DDCR death domain (amino acid residues from about 350 to about 420 in Figure 1 [SEQ ID NO 2]). It will be appreciated that reasonable persons of skill in the art may disagree, depending on the criteria used, concerning the exact "address" of the above-described DDCR domains. Thus, for example, the exact location of the DDCR ligand binding domain in Figure 1 [SEQ ID NO 2] may vary slightly (e.g., the address may "shift" by about 1 to 5 residues) depending on the criteria used to define the domain.

As indicated, nucleic acid molecules of the present invention which encode the DCCR polypeptide may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding a leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding

sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci., USA 86: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson et al., Cell 37: 767 (1984), for instance.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode for fragments, analogs or derivatives of the DCCR polypeptide. Variants may occur naturally, such as an allelic variant. Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Especially preferred among these are silent substitutions, additions and deletions, which do not alter

the properties and activities of the DDCR polypeptide or fragment thereof. Also especially preferred in this regard are conservative substitutions. Most highly preferred are nucleic acid molecules encoding the DCCR polypeptide having the amino acid sequence of Figure 1 (SEQ ID NO 2).

Further embodiments of the invention include isolated nucleic acid molecules that are at least 70% identical, and more preferably at least 80%, 90%, 95%, 97%, 98% or 99% identical, to the nucleic acid sequence shown in Figure 1 (SEQ ID NO 1), to fragments complementary thereto, or to fragments thereof selected from (1) a fragment that encodes the mature DDCR protein (i.e., the open reading frame), (2) a fragment that encodes the DDCR ligand binding domain, (3) a fragment that encodes the DDCR transmembrane domain, (4) a fragment that encodes the DDCR intracellular domain, and (5) a fragment that encodes the death domain.

The invention is further related to nucleic acid molecules that hybridize under stringent conditions to the above-described nucleic acid molecules. By "stringent conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 ug/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Polynucleotide assays

This invention is also related to the use of the DDCR polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of DDCR associated with a dysfunction will

provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from-under-expression over-expression or altered expression of DDCR or a soluble form thereof, such as, for example, tumors or autoimmune disease.

Individuals carrying mutations in the DDCR gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki et al., Nature, 324: 163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid encoding DDCR can be used to identify and analyze DDCR expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled DDCR RNA or alternatively, radiolabeled DDCR antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., Science, 230: 1242 (1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., Proc. Natl. Acad. Sci., USA, 85: 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by in situ analysis.

Chromosome assays

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step

in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a DDCR gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA the is used for in situ chromosome mapping using well known techniques for this purpose. Typically, in accordance with routine procedures for chromosome mapping, some trial and error may be necessary to identify a genomic probe that gives a good in situ hybridization signal.

In some cases, in addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include in situ hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence in situ hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This

technique can be used with cDNA as short as 50 or 60. For a review of this technique, see Verma et al., HUMAN CHROMOSOMES: A MANUAL OF BASIC TECHNIQUES, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, MENDELIAN INHERITANCE IN MAN, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Vectors and Host Cells

The present invention also relates to vectors which include DNA molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the

present invention. For instance, nucleic acid molecules may be introduced into host cells using well known techniques of infection, transduction, transfection, transvection and transformation. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

Thus, for instance, polynucleotides of the invention may be transfected into host cells with another, separate, polynucleotide encoding a selectable marker, using standard techniques for co-transfection and selection in, for instance, mammalian cells. In this case the polynucleotides generally will be stably incorporated into the host cell genome.

Alternatively, the polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. The vector construct may be introduced into host cells by the aforementioned techniques. Generally, a plasmid vector is introduced as DNA in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. Electroporation also may be used to introduce polynucleotides into a host. If the vector is a virus, it may be packaged in vitro or introduced into a packaging cell and the packaged virus may be transduced into cells. A wide variety of techniques suitable for making polynucleotides and for introducing polynucleotides into cells in accordance with this aspect of the invention are well known and routine to those of skill in the art. Such techniques are reviewed at length in Sambrook et al. cited above, which is illustrative of the many laboratory manuals that detail these techniques.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known

techniques for introducing DNA and RNA into cells. The vectors, in the case of phage and viral vectors also may be and preferably are introduced into cells as packaged or encapsidated virus by well known techniques for infection and transduction. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression. Such specific expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of vectors suitable to this aspect of the invention, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those of skill in the art.

The engineered host cells can be cultured in conventional nutrient media, which may be modified as appropriate for, inter alia, activating promoters, selecting transformants or amplifying genes. Culture conditions, such as temperature, pH and the like, previously used with the host cell selected for expression generally will be suitable for expression of polypeptides of the present invention as will be apparent to those of skill in the art.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques. In general, a DNA sequence for expression is joined to an expression vector by cleaving the DNA sequence and the expression vector with one or more restriction endonucleases and then joining the restriction fragments together using T4 DNA ligase. Procedures for restriction and ligation that can be used to this end are well known and routine to those of skill. Suitable procedures in this regard, and for constructing expression vectors using alternative techniques, which also are well known and routine to those skill, are set forth in great detail in Sambrook et al. cited elsewhere herein.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known

promoters. It will be understood that numerous promoters not mentioned are suitable for use in this aspect of the invention are well known and readily may be employed by those of skill in the manner illustrated by the discussion and the examples herein.

In general, expression constructs will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, in accordance with many commonly practiced procedures, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing E. coli and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast

cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

More particularly, the present invention also includes recombinant constructs, such as expression constructs, comprising one or more of the sequences described above. The constructs comprise a vector, such as a plasmid or viral vector, into which such a sequence of the invention has been inserted. The sequence may be inserted in a forward or reverse orientation. In certain preferred embodiments in this regard, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and there are many commercially available vectors suitable for use in the present invention.

The following vectors, which are commercially available, are provided by way of example. Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art for use in accordance with this aspect of the present invention. It will be appreciated that any other plasmid or vector suitable for, for example, introduction, maintenance, propagation or

expression of a polynucleotide or polypeptide of the invention in a host may be used in this aspect of the invention.

Promoter regions can be selected from any desired gene using vectors that contain a reporter transcription unit lacking a promoter region, such as a chloramphenicol acetyl transferase ("cat") transcription unit, downstream of restriction site or sites for introducing a candidate promoter fragment; i.e., a fragment that may contain a promoter. As is well known, introduction into the vector of a promoter-containing fragment at the restriction site upstream of the cat gene engenders production of CAT activity, which can be detected by standard CAT assays. Vectors suitable to this end are well known and readily available. Two such vectors are pKK232-8 and pCM7. Thus, promoters for expression of polynucleotides of the present invention include not only well known and readily available promoters, but also promoters that readily may be obtained by the foregoing technique, using a reporter gene.

Among known bacterial promoters suitable for expression of polynucleotides and polypeptides in accordance with the present invention are the E. coli lacI and lacZ and promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter. Among known eukaryotic promoters suitable in this regard are the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al. BASIC METHODS IN MOLECULAR BIOLOGY. (1986).

Constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Generally, recombinant expression vectors will include origins of replication, a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence, and a selectable marker to permit isolation of vector containing cells after exposure to the vector. Among suitable promoters are those derived from the genes that encode glycolytic enzymes such as 3-phosphoglycerate kinase ("PGK"), a-factor, acid phosphatase,

and heat shock proteins, among others. Selectable markers include the ampicillin resistance gene of E. coli and the trp1 gene of S. cerevisiae.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Polynucleotides of the invention, encoding the heterologous structural sequence of a polypeptide of the invention generally will be inserted into the vector using standard techniques so that it is operably linked to the promoter for expression. The polynucleotide will be positioned so that the transcription start site is located appropriately 3' to a ribosome binding site. The ribosome binding site will be 5' to the AUG that initiates translation of the polypeptide to be expressed. Generally, there will be no other open reading frames that begin with an initiation codon, usually AUG, and lie between the ribosome binding site and the initiating AUG. Also, generally, there will be a translation stop codon at the end of the polypeptide and there will be a polyadenylation signal and a transcription termination signal appropriately disposed at the 3' end of the transcribed region.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide.

The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

Suitable prokaryotic hosts for propagation, maintenance or expression of polynucleotides and polypeptides in accordance with the invention include Escherichia coli, Bacillus subtilis and Salmonella typhimurium. Various species of Pseudomonas, Streptomyces, and Staphylococcus are suitable hosts in this regard. Moreover, many other hosts also known to those of skill may be employed in this regard.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density,

where the selected promoter is inducible it is induced by appropriate means (e.g., temperature shift or exposure to chemical inducer) and cells are cultured for an additional period.

Cells typically then are harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can be employed for expression, as well. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblast, described in Gluzman et al., Cell 23: 175 (1981). Other cell lines capable of expressing a compatible vector include for example, the C127, 3T3, CHO, HeLa, human kidney 293 and BHK cell lines.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences that are necessary for expression. In certain preferred embodiments in this regard DNA sequences derived from the SV40 splice sites, and the SV40 polyadenylation sites are used for required non-transcribed genetic elements of these types.

The DDCR polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance

liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

DDCR polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of DDCR. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of DDCR by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

DDCR Polypeptides and Fragments

The invention further provides an isolated DDCR polypeptide having the amino acid sequence shown in Figure 1

[SEQ ID NO 2], or a fragment thereof. It will be recognized in the art that some amino acid sequence of DDCR can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains. In general, it is possible to replace residues which form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein.

Thus, the invention further includes variations of the DDCR protein which show substantial DDCR protein activity or which include regions of DDCR such as the protein fragments discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions (for example, substituting one hydrophilic residue for another, but not strongly hydrophilic for strongly hydrophobic as a rule). Small changes or such "neutral" amino acid substitutions will generally have little effect on activity.

Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the DDCR polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40

(1988).

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as polypeptides which have at least 70% similarity (preferably at least 70% identity) to the polypeptide of SEQ ID NO:2 and more preferably at least 90% similarity (more preferably at least 90% identity) to the polypeptide of SEQ ID NO:2 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

The presen- inventors have discovered that the DDCR polypeptide is a 428 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 30 to about 215 in Figure 1 [SEQ ID NO 2]. Second, the transmembrane domain was identified within residues from about 215 to about 240 in Figure 1 [SEQ ID NO 2]. Third, the intracellular domain was identified within residues from about 240 to about 428 in Figure 1 [SEQ ID NO 2]. Importantly, the intracellular domain includes a death domain at residues from about 350 to about 420. Further preferred fragments of the polypeptide shown in Figure 1 [SEQ ID NO 2] include the mature protein from residues about 30 to about 420.

Thus, the invention further provides DDCR polypeptide fragments selected from the mature DDCR protein the DDCR ligand binding domain, the DDCR transmembrane domain, the DDCR intracellular domain, and the DDCR death domain. As described in detail below, such fragments of DDCR can be used

to raise polyclonal and monoclonal antibody-agonists and antagonists capable of enhancing or inhibiting the DDCR ligand/receptor signaling pathway. Further, such fragments can be used in the yeast two-hybrid system to "capture" DDCR binding proteins which are also candidate agonist and antagonist according to the present invention. The yeast two hybrid system, which is discussed in more detail below, is described in Fields and Song, *Nature 340*:245-246 (1989). Further, the ligand binding domain in soluble form is itself useful as an antagonist capable of inhibiting DDCR signaling.

It will be appreciated by those of skill in the art that other DDCR fragments will also be useful for raising polyclonal and monoclonal antibodies according to the present invention. Such fragments include truncation mutants of the full length or mature DDCR polypeptide. Also preferred in this aspect of the invention are fragments characterized by structural or functional attributes of DDCR. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of DDCR.

Certain preferred regions in these regards are set out in Figure 3, and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in Figure 1. As set out in Figure 3, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions and coil-regions, Chou-Fasman alpha-regions, beta-regions and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophilic regions, Eisenberg alpha and beta amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf high

antigenic index regions.

Polypeptide assays

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of DDCR protein, or the soluble form thereof, in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of DDCR, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as an DDCR protein of the present invention, or a soluble form thereof, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to DDCR, or soluble form, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached a detectable reagent such as radioactive, fluorescent or enzymatic reagent, in this example horseradish peroxidase enzyme.

To carry out an ELISA assay, a sample is removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any DDCR proteins attached to the polystyrene dish. Unbound monoclonal antibody is washed out with buffer. The reporter

antibody linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to DDCR, or soluble receptor. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to DDCR through the primary and secondary antibodies, produces a colored reaction product. The amount of color developed in a given time period indicates the amount of DDCR protein, or soluble form, present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to DDCR, or soluble form, attached to a solid support and labeled DDCR and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of DDCR in the sample.

Therapeutics

The Tumor Necrosis Factor(TNF)family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (Goeddel, D.V. *et al.*, (1986) Tumor Necrosis factors: gene structure and biological activities. Cold Spring Harbor Symp. Quant. Biol. *51*, 597-609; Beutler, B., and Cerami, A. *Annu. Rev. Biochem.* *57*:505-518 (1988); Old, L.J., *Sci. Am. 258:* 59-75(1988); Fiers,W., *FEBS Lett. 285:*199-224(1991)). The TNF-family ligands induce such various cellular responses by binding to TNF-family receptors, including the DDCR of the present invention. Cells which express the DDCR polypeptide and have a potent cellular response to DDCR ligands include

lymphocytes, fibroblasts, macrophages, synovial cells, and epithelial cells. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis—programmed cell death—is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (Ameisen, J.C., *AIDS 8*:1197-1213 (1994); Krammer, P.H., *et al.*, *Curr. Opin. Immunol. 6*:279-289 (1994)).

Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, such as breast cancer, prostrate cancer, and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Thus, in one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DDCR polypeptide an effective amount of an

agonist capable of increasing DDCR mediated signaling. Preferably, DDCR mediated signaling is increased to treat a disease wherein decreased apoptosis is exhibited.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the D. polypeptide an effective amount of an antagonist capable of decreasing DDCR mediated signaling. Preferably, DDCR mediated signaling is decreased to treat a disease wherein increased apoptosis is exhibited.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in Science, volume 246, pages 181-296 (October 1989). For

example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992)).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DDCR polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the DDCR polypeptide can be contacted with either an endogenous or exogenously administed TNF-famly ligand.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor- β, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor supressors (p53), cytolytic T cells and antimetabolites. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and ß-amyloid peptide. (*Science 267*: 1457-

1458 (1995)). Further preferred agonist include polyclonal and monoclonal antibodies raised against the DDCR polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia, L.A., *et al.*, *Proc. Natl. Acad. Sci.* USA *88*:9292-9296 (1991); and Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992)). See, also, PCT Application WO 94/09137

Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *E1B*, Baculovirus *p35 and IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1*, *LMP-1*, African swine fever virus *LMW5-HL*, and Herpesvirus γ1 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and α-Hexachlorocyclohexane). Other antagonists include polyclonal and monoclonal antagonist antibodies raised against the DDCR polypeptides or a fragment thereof. Such antagonist antibodies raised against a TNF-family receptor are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992)); and Tartaglia, L.A., *et al.*, *Cell* 73:213-216 (1993)). See, also, PCT Application WO 94/09137

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of the receptor.

Further antagonist according to the present invention include soluble forms of DDCR, i.e., DDCR fragments that include the ligand binding domain from the extracellular region of the full length receptor. Such soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize DDCR mediated signaling by competing with the cell surface DDCR for binding to TNF-family ligands. Thus, soluble forms of the receptor that include the ligand binding domain are novel cytokines capable of inhibiting apoptosis induced by TNF-family ligands. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit apoptosis induced by Fas ligand (Hughes, D.P. and Crispe, I.N., *J. Exp. Med., 182*:1395-1401 (1995)).

As indicated polyclonal and monoclonal antibody agonist or antagonist according to the present invention can be raised according to the methods disclosed in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992)); Tartaglia, L.A., *et al., Cell 73*:213-216 (1993)), and PCT Application WO 94/09137. The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable

of binding an antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

Antibodies according to the present invention may be prepared by any of a variety of methods using DDCR immunogens of the present invention. As indicated, such DDCR immunogens include the full length DDCR polypeptide (which may or may not include the leader sequence) and DDCR polypeptide fragments such as the ligand binding domain, the transmembrane domain, the intracellular domain and the death domain.

In a preferred method, antibodies according to the present invention are mAbs. Such mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon et al., eds.), Elsevier, Amsterdam (1984)).

In general, such procedures involve immunizing an animal with a cell expressing the DDCR immunogen. The preferred animal for immunization is a mouse. Splenocytes of immunized animals are removed and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution. The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the DDCR immunogen.

For replication, the hybridoma cells of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* production makes this the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane-primed BALB/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Also intended within the scope of the present invention are humanized chimeric antibodies, produced using genetic constructs derived from hybridoma cells producing the mAbs described above. Methods for production of chimeric antibodies are known in the art. See, for review: Morrison, *Science*, 229:1202-1207 (1985); Oi et al., *BioTechniques* 4:214 (1986); see, also: Cabilly et al., U.S. Patent 4,816,567 (3/28/89); Taniguchi et al., EPO Patent Public. EP171496 (2/19/86); Morrison et al., EPO Patent Pub. EP173494 (3/5/86); Neuberger et al., PCT Pub. WO8601533 (3/13/86); Robinson et al., PCT Pub. WO 8702671 (5/7/87); Boulianne et al., *Nature* 312:643-646 (1984); Neuberger et al., *Nature* 314:268-270 (1985).

Proteins and other compounds which bind the DDCR domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeat two hybrid system (Fields and Song, *Nature 340*:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, J. *et al.*, *Cell 75*:791-803 (1993); Zervos, A.S. *et al.*, *Cell 72*:223-232 (1993)). Briefly, a domain of the DDCR polypeptide is used as bait for binding compounds. Positives are then selected by their ability to grow on plates lacking leucine, and then further tested for their ability to turn blue on plates with X-gal, as

previously described in great detail (Gyuris, J. *et al.*, *Cell* 75:791-803 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to either the DDCR ligand binding domain or to the DDCR intracellular domain. Such compounds are good candidate agonist and antagonist of the present invention. This system has been used previously to isolate proteins which bind to the intracellular domain of the p55 and p75 TNF receptors (WO 95/31544).

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, the DDCR ligand, TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40, CD27, CD30, 4-1BB, OX40 and nerve growth factor (NGF).

Representative therapeutic applications of the present invention are discussed in more detail below. The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of $CD4^+$ T-lymphocytes. Recent reports estimate the daily loss of $CD4^+$ T cells to be between 3.5 X 107 and 2 X 109 cells (Wei X., *et al.*, *Nature 373:* 117-122(1995)). One cause of $CD4^+$ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis. Indeed, HIV-induced apoptotic cell death has been demonstrated not only in vitro but also, more importantly, in infected individuals (Ameisen, J.C., *AIDS 8*:1197-1213 (1994); Finkel, T.H., and Banda, N.K., *Curr. Opin. Immunol. 6*:605-615(1995); Muro-Cacho, C.A. *et al.*, *J. Immunol. 154*:5555-5566 (1995)). Furthermore, apoptosis and CD4+ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner, T., *et al.*, *Nature 373:* 441-444 (1995); Gougeon, M.L., *et al.*, *AIDS Res. Hum. Retroviruses 9*:553-563 (1993)) and, apoptosis is not observed in those animal models

in which viral replication does not result in AIDS (Gougeon, M.L., *et al.*, *AIDS Res. Hum. Retroviruses 9*:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the de novo expression of FasL and that FasL mediates HIV-induced apoptosis (Badley, A.D., *et al.*, *J. Virol. 70*:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes (Badley, A.D., *et al.*, *J. Virol. 70*:199-206 (1996)). Thus, by the invention, a method for treating HIV individuals is provided which involves administering an antagonist of the present invention to reduce selective killing of CD4 T-lymphocytes. Modes of administration and dosages are discussed in detail below.

In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more that allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Agonist of the present invention are able to suppress the immune response to both allografts and xenografts because

lymphocytes activated and differentiated into effector cells will express the DDCR polypeptide, and thereby are susceptible to compounds which enhance apoptosis. Thus, the present invention further provides a method for creating immune privileged tissues. Antagonist of the invention can further be used in the treatment of Inflammatory Bowel Disease.

The agonist or antagonists described herein can be administered in vitro, ex vivo, or in vivo to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family ligand. In particular, by administration of an "effective amount" of an agonist or antagonists is intended an amount effective to enhance or inhibit DDCR mediated apoptosis. Of course, where apoptosis is to be enhanced, an agonist according to the present invention can be co-administered with a TNF-family ligand. One of ordinary skill will appreciate that effective amounts of an agonist or antagonist can be determined empirically and may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients. It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medial judgement. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the type and degree of the cellular response to be achieved; activity of the specific agonist or antagonist employed; the specific composition employed the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the agonist or antagonist; the

duration of the treatment; drugs used in combination or coincidental with the specific agonist or antagonist; and like factors well known in the medical arts.

For example, satisfactory results are obtained by oral administration of a antagonist or agonist at dosages on the order of from 0.05 to 10 mg/kg/day, preferably 0.1 to 7.5 mg/kg/day, more preferably 0.1 to 2 mg/kg/day, administered once or, in divided doses, 2 to 4 times per day. On administration parenterally, for example by i.v. drip or infusion, dosages on the order of from 0.01 to 5 mg/kg/day, preferably 0.05 to 1.0 mg/kg/day and more preferably 0.1 to 1.0 mg/kg/day can be used. Suitable daily dosages for patients are thus on the order of from 2.5 to 500 mg p.o., preferably 5 to 250 mg p.o., more preferably 5 to 100 mg p.o., or on the order of from 0.5 to 250 mg i.v., preferably 2.5 to 125 mg i.v. and more preferably 2.5 to 50 mg i.v.

Dosaging may also be arranged in a patient specific manner to provide a predetermined concentration of an agonist or antagonist in the blood, as determined by the RIA technique. Thus patient dosaging may be adjusted to achieve regular on-going trough blood levels, as measured by RIA, on the order of from 50 to 1000 ng/ml, preferably 150 to 500 ng/ml.

From above, pharmaceutical compositions are provided comprising an agonist or antagonist and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. Importantly, by co-administering an agonist and a TNF-family ligand, clinical side effects can be reduced by using lower doses of both the ligand and the agonist. It will be understood that the agonist can be "co-administered" either before, after, or simultaneously with the TNF-family ligand, depending on the exigencies of a

particular therapeutic application. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylceuulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compositions of the present invention may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the agonist or antagonist, it is desirable to slow the absorption

from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compounds are mixed with at least one item pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcerulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid,

certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and I) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

The active compounds can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isoptopyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame

oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In nonpressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 µm in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 µm.

Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition is preferably such that the active ingredient does not dissolve therein to any substantial extent. The pressurized composition may also contain a surface active agent. The surface active agent may be a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the agonist or antagonist with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the drugs.

The agonist or antagonist can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to the agonist or antagonist, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl choaes (lecithins), both natural and synthetic. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., *Methods in Cell Biology*, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq*.

Gene therapy

The DDCR polynucleotides, soluble form of the receptor polypeptides, agonists and antagonists that are polypeptides may be employed in accordance with the present invention by expression of such polypeptides in vivo, in treatment modalities often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide, such as a DNA or RNA, encoding a polypeptide ex vivo, and the engineered cells then can be provided to a patient to be treated with the polypeptide. For example, cells may be engineered ex vivo by

the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention. Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein.

Similarly, cells may be engineered in vivo for expression of a polypeptide in vivo by procedures known in the art. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct then may be isolated and introduced into a packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells in vivo and expression of the polypeptide in vivo. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention.

Retroviruses from which the retroviral plasmid vectors herein above mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

Such vectors well include one or more promoters for expressing the polypeptide. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller et al., Biotechniques 7: 980-990 (1989), or any other promoter (e.g., cellular promoters such

as eukaryotic cellular promoters including, but not limited to, the histone, RNA polymerase III, and ß-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention will be placed under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs herein above described); the ß-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, Y-2, Y-AM, PA12, T19-14X, VT-19-17-H2, YCRE, YCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, A., Human Gene Therapy 1: 5-14 (1990). The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a

host.

The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), herein referred to as "Sambrook."

All parts or amounts set out in the following examples are by weight, unless otherwise specified.

Unless otherwise stated size separation of fragments in the examples below was carried out using standard techniques of agarose and polyacrylamide gel electrophoresis ("PAGE") in

Sambrook and numerous other references such as, for instance, by Goeddel et al., Nucleic Acids Res. 8:4057 (1980).

Unless described othersie, ligations were accomplished using standard buffers, incubation temperatures and times, approximately equimolar amounds of the DNA fragments to be ligated and approximately 10 units of T4 DNA ligase ("ligase") per 0.5 ug of DNA.

Example 1: ***Expression in E. coli***

The following primers are used for expression of DDCR extracellular domain in E. coli 5' primer GCGCCATGGGGGCCCGGCGGCAG contains an NcoI site and 15 nucleotide starting from 290 nucleotide to 304 Figure 1. 3' primer GCGAAGCTTCTAGGACCCAGAACATCTGCC contains a HindIII site, a stop codon and 18 nucleotides complimentary to nucleotide from 822 to 840 in Figure 1. Vector is pQE60. The protein is not tagged.

Example 2: ***Expression in Mammalian Cells (CHO, COS and Others).***

Most of the vectors used for the transient expression of a given gene sequence in mammalian cells carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g. COS cells) which express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and

late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g. RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, also cellular signals can be used (e.g. human actin, promoter). Suitable expression vectors for use in practicing the present invention include , for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC67109). Mammalian host cells that could be used include, human Hela, 283, H9 and Jurkart cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1 African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells such as

Alternatively, a gene of interest can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Using this marker, the mammalian cells are grown in increasing amounts of methotrexate for selection and the cells with the highest resistance are selected These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, March 1985, 438-4470] plus a fragment of the CMV-enhancer (Boshart et al., Cell 41, 521-530 [1985]. Multiple cloning sites, e.g. with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors

contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 2A: Expression of extracellular soluble domain of DDCR in COS cells

The expression plasmid, DDCR HA, is made by cloning a cDNA encoding DDCR into the expression vector pcDNAI/Amp (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an E.coli origin of replication effective for propagation in E. coli and other prokaryotic cell; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

A DNA fragment encoding the entire DDCR precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson et al., Cell 37: 767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is as follows:

The DDCR cDNA of the deposit clone is amplified using primers that contained convenient restriction sites, much as described above regarding the construction of expression vectors for expression of DDCR in E. coli and S. fugiperda.

To facilitate detection, purification and character-

ization of the expressed DDCR, one of the primers contains a hemagglutinin tag ("HA tag") as described above.

Suitable primers include that following, which a used in this example; the 5' primer, 5' CGCGGATCCATGGAGGAGACGCAGCAG 3' contains the underlined BamHI site, an ATG start codon and 5 codons thereafter.

The 3' primer, containing the underlined XbaI site, stop codon, hemaggiutinin tag and last 14 nucleotide of 3' coding sequence (at the 3' end) has the following sequence; 5' GCGTCTAGATCAAAGCGTAGTCTGGGACGTCGTATGGGTACGGGCCGCGCTGCA 3'.

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and XbaI and then ligated. The ligation mixture is transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the DDCR-encoding fragment.

For expression of recombinant DDCP, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989).

Cells are incubated under conditions for expression of DDCR by the vector.

Expression of the DDCR HA fusion protein is detected by radiolabelling and immunoprecipitation, using methods described in, for example Harlow et al., ANTIBODIES: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing 35S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the

lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 28: Expression and purification of human DDCR using the CHO Expression System

The DNA sequence encoding DDCR in the deposited polynucleotide is amplified using PCR oligonucleotide primers specific to the amino acid carboxyl terminal sequence of the DDCR protein and to vector sequences 3' to the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5' and 3' sequences respectively.

The 5' oligonucleotide primer has the sequence 5'CGCGGATCCATGGAGGAGACGCAGCAG 3' containing the underlined BamHI restriction site, which encodes a start AUG, followed by 15 nucleotides of the DDCR coding sequence set out in Figure 1 beginning with the 1st base of the ATG codon.

The 3' primer has the sequence 5' CGCGGATCCTCACGGGCCGCGCTGCA 3' containing the underlined BamHI restriction site followed by 17 nucleotides complementary to the last 14 nucleotides of the DDCR coding sequence set out in Figure 1, plus the stop codon.

The restrictions sites are convenient to restriction enzyme sites in the CHO expression vectors PC4.

The amplified DDCR DNA and the vector PC4 both are digested with BamHI and the digested DNAs then ligated together. Insertion of the DDCR DNA into the BamHI

restricted vector placed the DDCR coding region downstream of and operably linked to the vector's promoter.

The ligation mixture is transformed into competent E. coli cells using standard procedures. Such procedures are described in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. (1989).

Example 3: Cloning and expression of the soluble extracellular domain of DDCR in a baculovirus expression system

The cDNA sequence encoding the soluble extracellular domain of DDCR protein in the deposited clone is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' CGCGGATCCCAGCCCCAGG TGTGACTGTGCCGGTGACTTCCACAAGAAG 3' containing the underlined Bam HI restriction enzyme site followed by Kozak sequence and a number of bases of the sequence of DDCR of Figure 1. Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding DDCR provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., J. Mol. Biol. 196: 947-950 (1987) is appropriately located in the vector portion of the construct.

The 3' primer has the sequence 5' GCGAGATCTAGTCTCGACCC AGAACATCTGCCTCC 3' containing the underlined XbaI restriction followed by nucleotides complementary to the DDCR nucleotide sequence set out in Figure 1, followed by the stop codon.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with

BamH1 and Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein F2.

The vector pA2GP is used to express the DDCR protein in the baculovirus expression system, using standard methods, such as those described in Summers et al. A MANUAL OF METHODS FOR BACULOVIRUS VECTORS AND INSECT CELL CULTURE PROCEDURES. Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the Autograph californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites. For an easy selection of recombinant virus the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter and is followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pA2GP, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow et al., Virology 170: 31-39, among others.

The plasmid is digested with the restriction enzymes Bam HI and XbaI and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V2".

Fragment F2 and the dephosphorylated plasmid V2 are ligated together with T4 DNA ligase. E.coli HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the

human DDCR gene by digesting DNA from individual colonies using Bam HI and XbaI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBacDDCR.

5 µg of the plasmid pBacDDCR is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84: 7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBacDDCR are mixed in a sterile well of a microtiter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained

plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. A clone containing properly inserted DDCR is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as V-DDCR.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-DDCR at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5 µCi of 35S-methionine and 5 µCi 35S cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

Example 4: Tissue distribution of DDCR expression

Northern blot analysis is carried out to examine the levels of expression of DDCR in human tissues, using methods described by, among others, Sambrook et al, cited above. Total cellular RNA samples are isolated with RNAzol™ B system (Biotecx Laboratories, Inc. 6023 South Loop East, Houston, TX 77033).

About 10µg of Total RNA is isolated from tissue samples. The RNA is size resolved by electrophoresis through a 1% agarose gel under strongly denaturing conditions. RNA is

blotted from the gel onto a nylon filter, and the filter then is prepared for hybridization to a detectably labeled polynucleotide probe.

As a probe to detect mRNA that encodes DDCR, the antisense strand of the coding region of the cDNA insert in the deposited clone is labeled to a high specific activity. The cDNA is labeled by primer extension, using the Prime-It kit, available from Stratagene. The reaction is carried out using 50 ng of the cDNA, following the standard reaction protocol as recommended by the supplier. The labeled polynucleotide is purified away from other labeled reaction components by column chromatography using a Select-G-50 column, obtained from 5-Prime - 3-Prime, Inc. of 5603 Arapahoe Road, Boulder, CO 80303.

The labeled probe is hybridized to the filter, at a concentration of 1,000,000 cpm/ml, in a small volume of 7% SDS, 0.5 M NaPO4, pH 7.4 at 65°C, overnight.

Thereafter the probe solution is drained and the filter is washed twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS. The filter then is dried and exposed to film at -70°C overnight with an intensifying screen.

Example 5: Gene therapeutic expression of human DDCR

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is plated in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature overnight. After 24 hours at room temperature, the flask is inverted - the chunks of tissue remain fixed to the bottom of the flask - and fresh media is added (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin). The tissue is then incubated at 37°C for approximately one

week. At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerges. The monolayer is trypsinized and scaled into larger flasks.

A vector for gene therapy is digested with restriction enzymes for cloning a fragment to be expressed. The digested vector is treated with calf intestinal phosphatase to prevent self-ligation. The dephosphorylated, linear vector is fractionated on an agarose gel and purified.

DDCR cDNA capable of expressing active DDCR, is isolated. The ends of the fragment are modified, if necessary, for cloning into the vector. For instance, 5' overhanging may be treated with DNA polymerase to create blunt ends. 3' overhanging ends may be removed using S1 nuclease. Linkers may be ligated to blunt ends with T4 DNA ligase.

Equal quantities of the Moloney murine leukemia virus linear backbone and the DDCR fragment are mixed together and joined using T4 DNA ligase. The ligation mixture is used to transform E. Coli and the bacteria are then plated onto agar-containing kanamycin. Kanamycin phenotype and restriction analysis confirm that the vector has the properly inserted gene.

Packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The vector containing the DDCR gene is introduced into the packaging cells by standard techniques. Infectious viral particles containing the DDCR gene are collected from the packaging cells, which now are called producer cells.

Fresh media is added to the producer cells, and after an appropriate incubation period media is harvested from the plates of confluent producer cells. The media, containing the infectious viral particles, is filtered through a Millipore filter to remove detached producer cells. The

filtered media then is used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the filtered media. Polybrene (Aldrich) may be included in the media to facilitate transduction. After appropriate incubation, the media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his, to select out transduced cells for expansion.

Engineered fibroblasts then may be injected into rats, either alone or after having been grown to confluence on microcarrier beads, such as cytodex 3 beads. The injected fibroblasts produce DDCR product, and the biological actions of the protein are conveyed to the host.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The disclosures of all patents, patent applications, and publications referred to herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: YU, GUO-LIANG
NI, JIAN
GENTZ, REINER L

(ii) TITLE OF INVENTION: DEATH DOMAIN CONTAINING RECEPTOR

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068-1739

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Ferraro, Gregory D
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-553

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1783 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 198..1481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CATGGGTGGG GGTGGGGGCG CTGCTGGATT CCTGCTCTGG TGGAAGGGAA ACTTGTGAGG 60

GGCTGGTAAG CGCCCCCTCC GAAGCCTGGT GTGTGCGCGG GGGGAAGGAA GTTAGTTTCC 120

TCTCCACCCA TGGGCACCCC TTCTGCCCGG GGCCTGGGAA GTGGGCTGCT CTGTGGGCAA 180

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ATGCTGGGGC CTCTGAA ATG GAG GAG ACG CAG CAG GGA GAG GCC CCA CGT      230
                   Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg
                     1               5                  10

GGG CAG CTG CGC GGA GAG TCA GCA GCA CCT GTC CCC CAG GCG CTC CTC      278
Gly Gln Leu Arg Gly Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu
            15                  20                  25

CTG GTG CTG CTG GGG GCC CGG GCC CAG GGC GGC ACT CGT AGC CCC AGG      326
Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
        30                  35                  40

TGT GAC TGT GCC GGT GAC TTC CAC AAG AAG ATT GGT CTG TTT TGT TGC      374
Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
    45                  50                  55

AGA GGC TGC CCA GCG GGG CAC TAC CTG AAG GCC CCT TGC ACG GAG CCC      422
Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
60                  65                  70                  75

TGC GGC AAC TCC ACC TGC CTT GTG TGT CCC CAA GAC ACC TTC TTG GCC      470
Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
                80                  85                  90

TGG GAG AAC CAC CAT AAT TCT GAA TGT GCC CGC TGC CAG GCC TGT GAT      518
Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
            95                  100                 105

GAG CAG GCC TCC CAG GTG GCG CTG GAG AAC TGT TCA GCA GTG GCC GAC      566
Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
        110                 115                 120

ACC CGC TGT GGC TGT AAG CCA GGC TGG TTT GTG GAG TGC CAG GTC AGC      614
Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
    125                 130                 135

CAA TGT GTC AGC AGT TCA CCC TTC TAC TGC CAA CCA TGC CTA GAC TGC      662
Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
140                 145                 150                 155

GGG GCC CTG CAC CGC CAC ACA CGG CTA CTC TGT TCC CGC AGA GAT ACT      710
Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
                160                 165                 170

GAC TGT GGG ACC TGC CTG CCT GGC TTC TAT GAA CAT GGC GAT GGC TGC      758
Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
            175                 180                 185

GTG TCC TGC CCC ACG AGC ACC CTG GGG AGC TGT CCA GAG CGC TGT GCC      806
Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
        190                 195                 200

GCT GTC TGT GGC TGG AGG CAG ATG TTC TGG GTC CAG GTG CTC CTG GCT      854
Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
    205                 210                 215

GGC CTT GTG GTC CCC CTC CTG CTT GGG GGC ACC CTT GAC CTA CAC ATA      902
Gly Leu Val Val Pro Leu Leu Leu Gly Gly Thr Leu Asp Leu His Ile
220                 225                 230                 235

CCG CCA CTG CTG GCT CAC AAG CCC CTG GTT ACT GCA GAT GAA GCT GGG      950
Pro Pro Leu Leu Ala His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
                240                 245                 250

ATG GAG GCT CTG AAC CCA CCA CCG GGC ACC CAT CTG TCA CCC TTG GAC      998
Met Glu Ala Leu Asn Pro Pro Pro Gly Thr His Leu Ser Pro Leu Asp
            255                 260                 265
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AGC GCC CAC ACC CTT CTA GCA CCT CCT GAC AGC AGT GAG AAG ATC TGC      1046
Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
        270                 275                 280

ACC GTC CAG TTG GTG GGT AAC AGC TGG ACC CCT GGC TAC CCC GAG ACC      1094
Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
    285                 290                 295

CAG GAG GCG CTC TGC CCG CAG GTG ACA TGG TCC TGG GAC CAG TTG CCC      1142
Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
300                 305                 310                 315

AGC AGA GCT CTT GGC CCC GCT GCT GCG CCC ACA CTC TCG CCA GAG TCC      1190
Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
                320                 325                 330

CCA GCC GGC TCG CCA GCC ATG ATG CTG CAG CCG GGC CCG CAG CTC TAC      1238
Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
            335                 340                 345

GAC GTG ATG GAC GCG GTC CCA GCG CGG CGC TGG AAG GAG TTC GTG CGC      1286
Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
        350                 355                 360

ACG CTG GGG CTG CGC GAG GCA GAG ATC GAA GCC GTG GAG GTG GAG ATC      1334
Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
    365                 370                 375

GGC CGC TTC CGA GAC CAG CAG TAC GAG ATG CTC AAG CGC TGG CGC CAG      1382
Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
380                 385                 390                 395

CAG CAG CCC GCG GGC CTC GGA GCC GTT TAC GCG GCC CTG GAG CGC ATG      1430
Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
                400                 405                 410

GGG CTG GAC GGC TGC GTG GAA GAC TTG CGC AGC CGC CTG CAG CGC GGC      1478
Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
            415                 420                 425

CCG TGACACGGCG CCCACTTGCC ACCTAGGCGC CCTGGTGGCC CTTGCAGAAG           1531
Pro

CCCTAAGTAC GGTTACTTAT GCGTGTAGAC ATTTTATGTC ACTTATTAAG CCGCTGGCAC    1591

GGCCCTGCGT AGCAGCACCA GCCGGCCCCA CCCCTGCTCG CCCCTATCGC TCCAGCCAAG    1651

GCGAAGAAGC ACGAACGAAT GTCGAGAGGG GGTGAAGACA TTTCTCAACT TCTCGGCCGG    1711

AGTTTGGCTG AGATCGCGGT ATTAAATCTG TGAAAGAAAA CAAAACAAAA CAAAAAAAAA    1771

AAAAAAAAAA AA                                                        1783
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg Gly Gln Leu Arg Gly
  1               5                  10                  15
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Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu Leu Val Leu Leu Gly
20 25 30

Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg Cys Asp Cys Ala Gly
35 40 45

Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys Arg Gly Cys Pro Ala
50 55 60

Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro Cys Gly Asn Ser Thr
65 70 75 80

Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala Trp Glu Asn His His
85 90 95

Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp Glu Gln Ala Ser Gln
100 105 110

Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp Thr Arg Cys Gly Cys
115 120 125

Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser Gln Cys Val Ser Ser
130 135 140

Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys Gly Ala Leu His Arg
145 150 155 160

His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr Asp Cys Gly Thr Cys
165 170 175

Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys Val Ser Cys Pro Thr
180 185 190

Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala Ala Val Cys Gly Trp
195 200 205

Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala Gly Leu Val Val Pro
210 215 220

Leu Leu Leu Gly Gly Thr Leu Asp Leu His Ile Pro Pro Leu Leu Ala
225 230 235 240

His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly Met Glu Ala Leu Asn
245 250 255

Pro Pro Pro Gly Thr His Leu Ser Pro Leu Asp Ser Ala His Thr Leu
260 265 270

Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys Thr Val Gln Leu Val
275 280 285

Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr Gln Glu Ala Leu Cys
290 295 300

Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro Ser Arg Ala Leu Gly
305 310 315 320

Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser Pro Ala Gly Ser Pro
325 330 335

Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr Asp Val Met Asp Ala
340 345 350

Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg Thr Leu Gly Leu Arg
355 360 365

Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile Gly Arg Phe Arg Asp
370 375 380

```
Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln Gln Gln Pro Ala Gly
385                 390                 395                 400

Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met Gly Leu Asp Gly Cys
                405                 410                 415

Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly Pro
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1               5                   10                  15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
            20                  25                  30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Asn Leu
        35                  40                  45

Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro Pro
    50                  55                  60

Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro Asp
65                  70                  75                  80

Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His Phe
                85                  90                  95

Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Phe Gly His Gly Leu
            100                 105                 110

Glu Val Glu Ile Asn Cys Thr Arg Thr Asn Thr Lys Cys Arg Cys Lys
        115                 120                 125

Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp Pro Cys
    130                 135                 140

Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr Ser Asn
145                 150                 155                 160

Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp Leu Cys
                165                 170                 175

Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg Lys Glu
            180                 185                 190

Val Cys Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly Ser His
        195                 200                 205

Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu Ser Asp
    210                 215                 220

Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met Thr Leu
225                 230                 235                 240
```

Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu Ala Lys
245 250 255

Ile Asp Glu Ile Asp Asn Asp Asn Val Gln Asp Thr Ala Glu Gln Lys
260 265 270

Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys Glu Ala
275 280 285

Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys Thr Leu
290 295 300

Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser Asp Ser
305 310 315 320

Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
325 330

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 453 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
210 215 220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225 230 235 240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
245 250 255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
260 265 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
275 280 285

Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys Pro
290 295 300

Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly Ala
305 310 315 320

Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn Pro
325 330 335

Leu Gln Lys Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp Thr Asp
340 345 350

Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro Leu Arg
355 360 365

Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu Ile Asp
370 375 380

Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln Tyr Ser
385 390 395 400

Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala Thr Leu
405 410 415

Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly Cys Leu
420 425 430

Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro Pro Ala
435 440 445

Pro Ser Leu Leu Arg
450

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCGCCATGGG GGCCCGGCGG CAG 23

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCGAAGCTTC TAGGACCCAG AACATCTGCC 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGCGGATCCA TGGAGGAGAC GCAGCAG 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGGATCCT CACGGGCTGC GCTGCA 26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCGGATCCC AGCCCCAGGT GTGACTGTGC CGGTGACTTC CACAAGAAG 49

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCGAGATCTA GTCTGGACCC AGAACATCTG CCTCC 35

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGCGGATCCA TGGAGGAGAC GCAGCAG 27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCGTCTAGAT CAAGCGTAGT CTGGACGTC GTATGGGTAC GGGCCGCGCT GCA 53

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a polynucleotide having at least 70% identity to a member selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide comprising amino acid 1 to amino acid 428 set forth in SEQ ID NO:2; and

(b) a polynucleotide which is complementary to the polynucleotide of (a).

2. The nucleic acid molecule of claim 1 wherein the polynucleotide is DNA.

3. The nucleic acid molecule of claim 1 wherein the polynucleotide is RNA.

4. The nucleic acid molecule of claim 1 wherein the polynucleotide is genomic DNA.

5. The nucleic acid molecule of claim 2 which encodes a polypeptide comprising amino acid 1 to 428 of SEQ ID NO:2.

6. An isolated nucleic acid molecule comprising a polynucleotide having at least 70% identity to a member selected from the group consisting of:

(a) a polynucleotide which encodes a mature polypeptide having the amino acid sequence expressed by the human cDNA contained in ATCC Deposit No. 97456; and

(b) a polynucleotide which is complementary to the polynucleotide of (a).

7. The nucleic acid molecule of claim 1 comprising the sequence as set forth in SEQ ID NO:1 from nucleotide 198 to nucleotide 1479.

8. The nucleic acid molecule of claim 1 comprising the sequence as set forth in SEQ ID NO:1 from nucleotide 1 to 1780.

9. A vector comprising the nucleic acid molecule of claim 1.

10. A host cell comprising the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing a cell comprising: genetically engineering the cell with the vector of Claim 9 to thereby express the polypeptide encoded by the DNA contained in the vector.

13. An isolated nucleic acid molecule comprising a polynucleotide encoding a DDCR polypeptide having the amino acid sequence shown in SEQ ID NO 2, or a fragment of said polypeptide.

14. An isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO 1, or a fragment thereof.

15. The isolated nucleic acid molecule of claim 14, wherein said fragment comprises an open reading frame whose initiation codon is at position 198-200 of the nucleotide sequence shown in SEQ ID NO 1.

16. The isolated nucleic acid molecule of claim 14, wherein said fragment comprises a polynucleotide encoding the DDCR ligand binding domain.

17. The isolated nucleic acid molecule of claim 14, wherein said fragment comprises a polynucleotide encoding the DDCR transmembrane domain.

18. The isolated nucleic acid molecule of claim 14, wherein said fragment comprises a polynucleotide encoding the DDCR intracellular domain.

19. The isolated nucleic acid molecule of claim 14, wherein said fragment comprises a polynucleotide encoding the DDCR death domain.

20. An isolated polypeptide selected from the group consisting of:

(a) a polypeptide having the amino acid sequence set forth in SEQ ID NO:2; and

(b) a polypeptide which is at least 70% identical to the polypeptide of (a).

21. An isolated polypeptide having the amino acid sequence shown in SEQ ID NO 2, or a fragment thereof.

22. The isolated polypeptide of claim 21, wherein said fragment comprises the DDCR ligand binding domain.

23. The isolated polypeptide of claim 21, wherein said fragment comprises the DDCR transmembrane domain.

24. The isolated polypeptide of claim 21, wherein said fragment comprises the DDCR intracellular domain.

25. The isolated polypeptide of claim 21, wherein said fragment comprises the DDCR death domain.

26. A method for enhancing apoptosis induced by a TNF-family ligand comprising administering to a cell which expresses the DDCR polypeptide an effective amount of an agonist capable of increasing DDCR mediated signaling.

27. The method of claim 26, wherein DDCR mediated signaling is increased to treat a disease exhibiting decreased apoptosis.

28. The method of claim 27, wherein said disease is selected from cancer, an autoimmune disorder, viral infection, or graft v. host disease.

29. The method of claims 28, wherein said cancer is selected from follicular lymphomas, carcinomas with p53 mutations, or hormone-dependent tumors.

30. The method of claim 29, wherein said hormone-dependent tumors are selected from breast cancer, prostrate cancer, or ovarian cancer.

31. The method of claim 28, wherein said autoimmune disorder is selected from systemic lupus erythematosus or immune-related glomerulonephritis.

32. The method of claim 28, wherein said viral infection is selected from herpesvirus, poxvirus, or adenovirus infection.

33. A method for inhibiting apoptosis induced by a TNF-family ligand comprising administering to a cell which expresses the DDCR polypeptide an effective amount of an antagonist capable of decreasing DDCR mediated signaling.

34. The method of claim 33, wherein DDCR mediated signaling is decreased to treat a disease exhibiting increased apoptosis.

35. The method of claim 34, wherein said disease is selected from AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia, or anorexia.

36. The method of claim 35, wherein said neurodegenerative disorder is selected from Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, or Cerebellar degeneration.

37. The method of claim 35, wherein said myelodysplastic syndrome is aplastic anemia.

38. The method of claim 35, wherein said ischemic injury is caused by myocardial infarction, stroke or reperfusion.

39. The method of claim 35, wherein said toxin-induced liver disease is caused by alcohol.

40. The method of claim 26, wherein said agonist is selected from TNF family ligand peptide fragments, transforming growth factor β peptide fragments, neurotransmitters, tumor supressors, cytolytic T cells, antimetabolites, chemotherapeutic drugs, anti-DDCR polyclonal antibodies, or anti-DDCR monoclonal antibodies.

41. The method of claim 40, wherein said neurotransmitter is selected from glutamate, dopamine, or *N*-methyl-D-aspartate.

42. The method of claim 40, wherein said chemotherapeutic drug is selected from cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate, or vincristine.

43. The method of claim 33, wherein said antagonist is selected from CD40 ligand neutral amino acids, zinc, estrogen, androgens, calpain inhibitors, cysteine protease inhibitors, tumor promoters, anti-DDCR polyclonal antibodies, anti-DDCR monoclonal antibodies, or a soluble polypeptide comprising the DDCR extracellular domain.

44. The method of claim 43, wherein said tumor promoter is selected from PMA, Phenobarbital, or α-Hexachlorocyclohexane.

45. A screening method for determining whether a candidate agonist is capable of enhancing a cellular response to a TNF-family ligand, comprising:

(a) contacting cells which express the DDCR polypeptide with a candidate agonist and a TNF-family ligand;

(b) assaying a cellular response;

(c) comparing said cellular response to a standard cellular response, said standard being assayed when contact is made with the ligand in absence of the candidate agonist; whereby,

an increased cellular response over the standard indicates that the candidate agonist is a potentiator of DDCR-mediated signaling.

46. A screening method for determining whether a candidate antagonist is capable of inhibiting a cellular response to a TNF-family ligand, comprising:

(a) contacting cells which express the DDCR polypeptide with a candidate antagonist and a TNF-family ligand;

(b) assaying a cellular response;

(c) comparing said cellular response to a standard cellular response, said standard being assayed when contact is made with the ligand in absence of the candidate antagonist; whereby,

a decreased cellular response over said standard indicates that the candidate antagonist is an inhibitor of DDCR-mediated signaling.

47. The method of claim 45, wherein said cell is contacted with an endogenous TNF-family ligand.

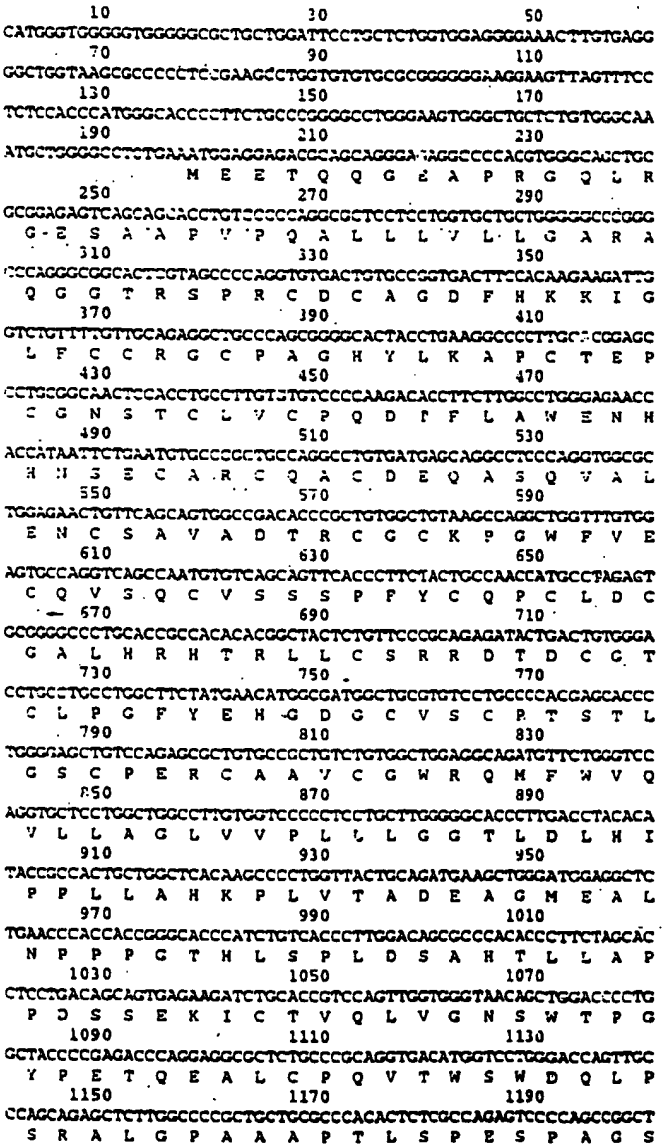
48. The method of claim 46, wherein said cell is contacted with an endogenous TNF-family ligand.

49. The method of claim 45, wherein said cell is contacted with an exogenously administed TNF-family ligand.

50. The method of claim 46, wherein said cell is contacted with an exogenously administed TNF-family ligand.

ABSTRACT OF THE DISCLOSURE

The invention concerns a novel member of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding death domain containing (DDCR) polypeptides. DDCR polypeptides are also provided as are screening methods for identifying agonist and antagonist capable of potentiating or inhibiting DDCR-mediated signaling. The invention further concerns therapeutic methods for treating diseases associated with disregulation of apoptosis.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G E S A A P V P Q A L L L V L L G A R A
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Q G G T R S P R C D C A G D F H K K I G
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L F C C R G C P A G H Y L K A P C T E P
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C G N S T C L V C P Q D T F L A W E N H
490 510 530
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H N S E C A R C Q A C D E Q A S Q V A L
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E N C S A V A D T R C G C K P G W F V E
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G A L H R H T R L L C S R R D T D C G T
730 750 770
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C L P G F Y E H G D G C V S C P T S T L
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G S C P E R C A A V C G W R Q M F W V Q
850 870 890
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V L L A G L V V P L L L G G T L D L H I
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970 990 1010
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N P P P G T H L S P L D S A H T L L A P
1030 1050 1070
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P D S S E K I C T V Q L V G N S W T P G
1090 1110 1130
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Y P E T Q E A L C P Q V T W S W D Q L P
1150 1170 1190
CCAGCAGAGGCTCTTGGCCCCGCTGCTGCGCCCACACTCTCGCCAGAGTCCCCAGCCGGCT
S R A L G P A A A P T L S P E S P A G S

FIGURE 1

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```
     1210                1230                1250
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  P  A  M  M  L  Q  P  G  P  Q  L  Y  D  V  M  D  A  V  P  A
     1270                1290                1310
CGCGGCGCTGGAAGGAGTTCGTGCGCACGCTGGGGCTGCGCGAGGCAGAGATCGAAGCCG
  R  R  W  K  E  F  V  R  T  L  G  L  R  E  A  E  I  E  A  V
     1330                1350                1370
TGGAGGTGGAGATCGGACGCTTCCGAGACCAGCAGTACGAGATGCTCAAGCGCTGGCGCC
  E  V  E  I  G  R  F  R  D  Q  Q  Y  E  M  L  K  R  W  R  Q
     1390                1410                1430
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  Q  Q  P  A  G  L  G  A  V  Y  A  A  L  E  R  M  G  L  D  G
     1450                1470                1490
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  C  V  E  D  L  R  S  R  L  Q  R  G  P
     1510                1530                1550
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     1570                1590                1610
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     1630                1650                1670
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FIGURE 1 (CONT.)

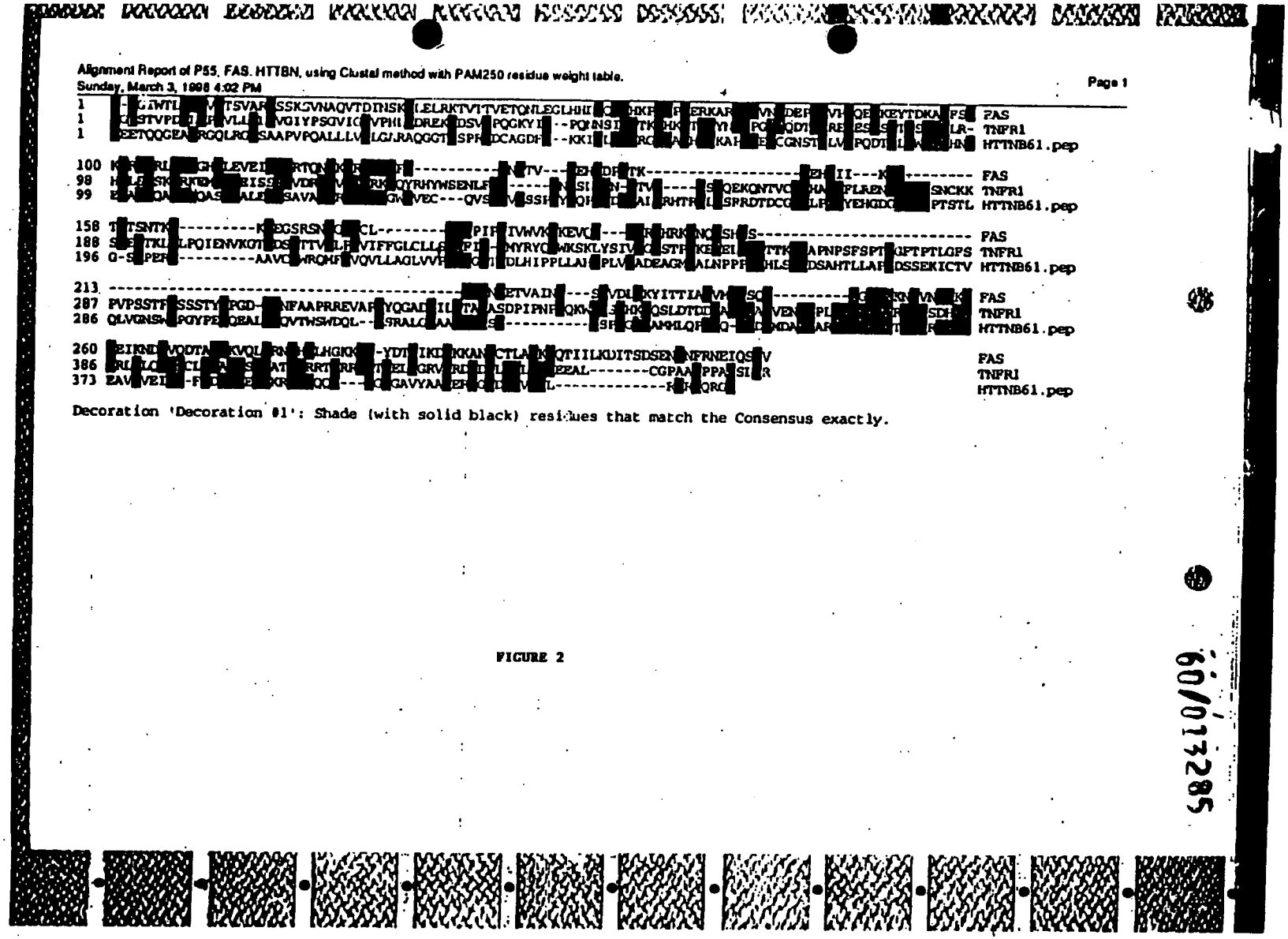

FIGURE 2

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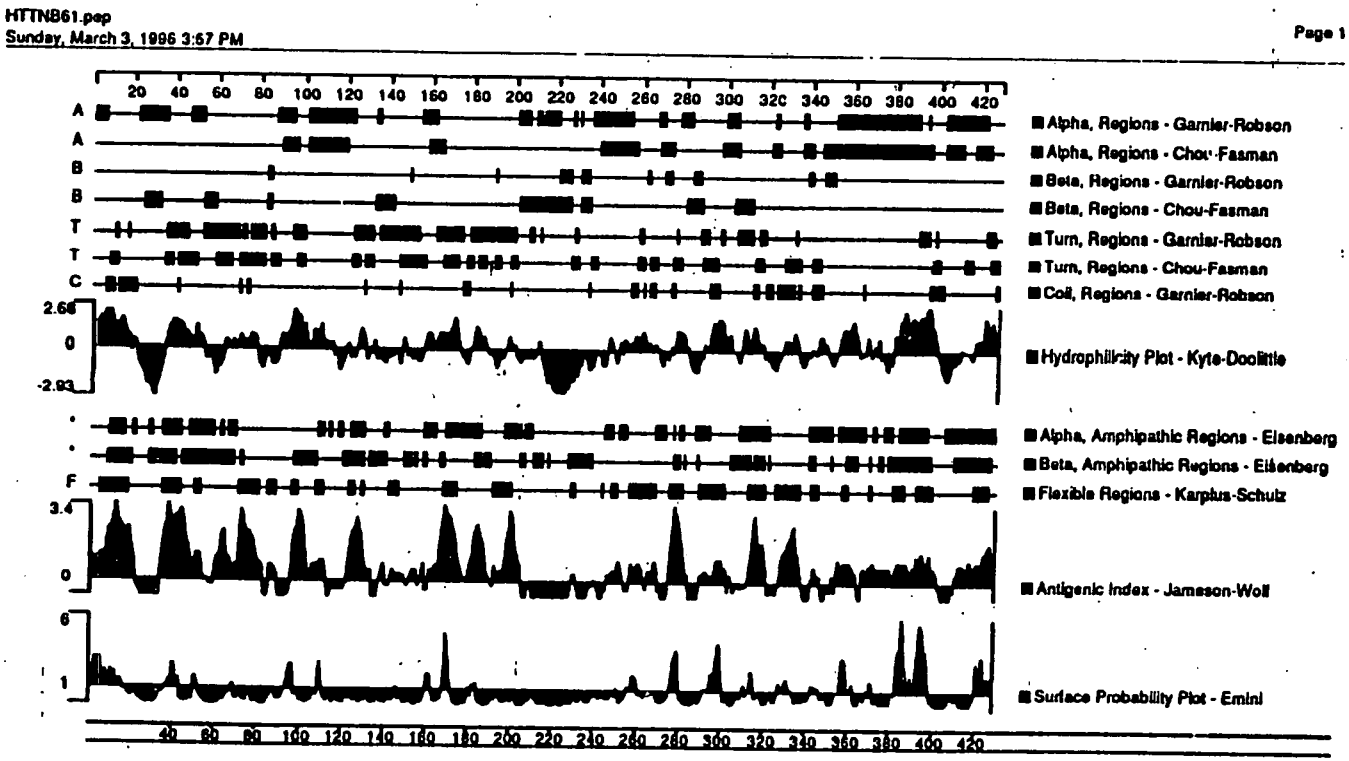

FIGURE 3

60/013285

MAIL ROOM
MAR
12 6

60/013285

"EXPRESS MAIL CERTIFICATE"

"Express Mail" Mailing Number | Date of Deposit

I hereby certify that this application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

(Typed or Printed Name of Person Mailing Application)

(Signature of Person Mailing Application)

U.S. PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a Provisional Application for Patent under CFR 1.53 (b) (2).

	Docket No.	PF267PP	Type a plus sign (+) inside this box →	+

INVENTOR (S) / APPLICANTS (S)

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TITLE OF THE INVENTION (280 characters max.)

Death Domain Containing Receptor

CORRESPONDENCE ADDRESS

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ENCLOSED APPLICATION PARTS (check all that apply)

- [X] Specification Number of Pages -74-
- [X] Drawings Number of Sheets -4-
- [ ] Small Entity Statement
- [X] Other Specify -Sequence Listing+ diskette

METHOD OF PAYMENT (check one)

- [ ] A check or money order is enclosed to cover the Provisional filing fee
- [X] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 08-3425

PROVISIONAL FILING FEE AMOUNT ($)	$130.00

The invention was made by an agency of the U.S. Government or under a contract with an agency of the United States Government

- [X] No.
- [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully Submitted,

Signature Robert H. Benson Date: March 12, 1996

Typed or Printed Name Robert H. Benson Registration No. (If appropriate) 30,446

[X] Additional inventors are being named on separately numbered sheet attached hereto.

PROVISIONAL APPLICATION FILING ONLY

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ANNEX TO
PROVISIONAL APPLICATION COVER SHEET

Docket No. PF267PP	Title: Death Domain Containg Receptor

ADDITIONAL INVENTORS

Last Name	First Name	Middle Initial	Residence (*City and Either State or Foreign Country*)
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Exhibit A

Appendix of Claims on Appeal

34. (Previously presented) Isolated nucleic acid encoding Apo-3 polypeptide comprising amino acid residues 1 to 417, 25 to 417, 25 to 198, or 338 to 417 of SEQ ID NO: 6, or a biologically active variant thereof.

36. (Original) A vector comprising the nucleic acid of claim 34.

37. (Original) The vector of claim 36 operably linked to control sequences recognized by a host cell transformed with the vector.

38. (Original) A host cell comprising the vector if claim 36.

39. (Previously presented) A process of producing Apo-3 polypeptide comprising culturing the host cell of claim 38 and isolating said polypeptide.

Exhibit C

Death Domain Containing Receptors

Background of the Invention

Field of the Invention

The present invention relates to a novel member of the tumor necrosis factor family of receptors. More specifically, isolated nucleic acid molecules are provided encoding human Death Domain Containing Receptors (DR3 and DR3-V1). Death Domain Containing Receptor polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR3 activity.

Related Art

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intra-cellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-lBBL, OX40L and nerve growth factor (NGF). The

TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-IBB, OX40, low affinity p75 and NGF-receptor (Meager, A., *Biologicals, 22*:291-295 (1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., *supra*).

Considerable insight into the essential functions of several members of the TNF receptor family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., *et al., Nature 356*:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglobulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. *et al., Science 259*:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. *et al., Cell 69*:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmune disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., *Science 264*:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (p55) and Fas was reported as the "death domain," which is responsible for

transducing signals for programmed cell death (Tartaglia *et al., Cell 74*:845 (1993)).

Apoptosis, or programmed cell death, is a physiologic process essential to the normal development and homeostasis of multicellular organisms (H. Steller, *Science* 267, 1445-1449 (1995)). Derangements of apoptosis contribute to the pathogenesis of several human diseases including cancer, neurodegenerative disorders, and acquired immune deficiency syndrome (C.B. Thompson, *Science* 267, 1456-1462 (1995)). Recently, much attention has focused on the signal transduction and biological function of two cell surface death receptors, Fas/APO-1 and TNFR-1 (J.L. Cleveland, *et al., Cell* 81, 479-482 (1995); A. Fraser, *et al., Cell* 85, 781-784 (1996); S. Nagata, *et al., Science* 267, 1449-56 (1995)). Both are members of the TNF receptor family which also include TNFR-2, low affinity NGFR, CD40, and CD30, among others (C.A. Smith, *et al., Science 248*, 1019-23 (1990); M. Tewari, *et al.,* in *Modular Texts in Molecular and Cell Biology* M. Purton, Heldin, Carl, Ed. (Chapman and Hall, London, 1995). While family members are defined by the presence of cysteine-rich repeats in their extracellular domains, Fas/APO-1 and TNFR-1 also share a region of intracellular homology, appropriately designated the "death domain", which is distantly related to the Drosophila suicide gene, reaper (P. Golstein, *et al., Cell 81*, 185-6 (1995); K. White *et al., Science* 264, 677-83 (1994)). This shared death domain suggests that both receptors interact with a related set of signal transducing molecules that, until recently, remained unidentified. Activation of Fas/APO-1 recruits the death domain-containing adapter molecule FADD/MORT1 (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M. P. Boldin, *et al., J. Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)), which in turn binds and presumably activates FLICE/MACH1, a member of the ICE/CED-3 family of pro-apoptotic proteases (M. Muzio *et al., Cell 85*, 817-827 (1996); M.P. Boldin, *et al., Cell 85*, 803-815 (1996)). While the central role of Fas/APO-1 is to trigger cell death, TNFR-1 can signal an array of diverse biological activities-many of which stem from its ability to activate NF-kB (L.A. Tartaglia, *et al., Immunol Today 13*, 151-3

(1992)). Accordingly, TNFR-1 recruits the multivalent adapter molecule TRADD, which like FADD, also contains a death domain (H. Hsu, *et al., Cell 81*, 495-504 (1995); H. Hsu, *et al., Cell 84*, 299-308 (1996)). Through its associations with a number of signaling molecules including FADD, TRAF2, and RIP, TRADD can signal both apoptosis and NF-kB activation (H. Hsu, *et al., Cell 84*, 299-308 (1996); H. Hsu, *et al., Immunity 4*, 387-396 (1996)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF receptor family.

Summary of the Invention

The present invention provides for isolated nucleic acid molecules comprising nucleic acid sequences encoding the amino acid sequences shown in FIG. 1 (SEQ ID NO:2) and FIG. 2 (SEQ ID NO:4) or the amino acid sequence encoding the cDNA clones deposited in a bacterial host as ATCC Deposit No. 97456 on March 1, 1996 and ATCC Deposit No. ___ on October 10, 1996.

The present invention also provides vectors and host cells for recombinant expression of the nucleic acid molecules described herein, as well as to methods of making such vectors and host cells and for using them for production of DR3 or DR3 Variant 1 (DR3-V1) (formerly named DDCR) polypeptides or peptides by recombinant techniques.

The invention further provides an isolated DR3 or DR3-V1 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The present invention also provides diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR3 or DR3-V1 protein. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-

expression of DR3 or DR3-V1, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors.

Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes. Cellular response to TNF-family ligands include not only normal physiological responses, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes. Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers, autoimmune disorders, viral infections, inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury, toxin-induced liver disease, septic shock, cachexia and anorexia.

Thus, the invention further provides a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR3 polypeptide an effective amount of an agonist capable of increasing DR3 mediated signaling. Preferably, DR3 mediated signaling is increased to treat a disease wherein decreased apoptosis is exhibited.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR3 polypeptide an effective amount of an antagonist capable of decreasing DR3 mediated signaling. Preferably, DR3 mediated signaling is decreased to treat a disease wherein increased apoptosis is exhibited.

Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail

below. Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR3 or DR3-V1 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By the invention, a cell expressing the DR3 or DR3-V1 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

Brief Description of the Figures

FIG. 1A-B shows the nucleotide and deduced amino acid sequence of DR3-V1. It is predicted that amino acids 1 - 35 constitute the signal peptide, amino acids 36-212 constitute the extracellular domain, amino acids 213-235 constitute the transmembrane domain, amino acids 236-428 constitute the intracellular domain, and amino acids 353-419 the death domain.

FIG. 2 shows the nucleotide and deduced amino acid sequence of DR3. It is predicted that amino acids 1 - 24 constitute the signal peptide, amino acids 25-201 constitute the extracellular domain, amino acids 202-224 constitute the transmembrane domain, amino acids 225-417 constitute the intracellular domain, and amino acids 342-408 constitute the death domain.

FIG. 3 shows the regions of similarity between the amino acid sequences of the DR3-V1, human tumor necrosis factor receptor 1, and Fas receptor [SEQ ID NOs:5 and 6].

FIG. 4 shows an analysis of the DR3-V1 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic

regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues1-22, 33-56, 59-82, 95-112, 122-133, 161-177, 179-190, 196-205 in Figure 1 correspond to the shown highly antigenic regions of the DR3-V1 protein.

Detailed Description of the Preferred Embodiments

The present invention provides isolated nucleic acid molecules comprising a nucleic acid sequence encoding the DR3-V1 or DR3 polypeptide whose amino acid sequence is shown in FIG. 1 [SEQ ID NO:2] and FIG. 2 [SEQ ID NO:4], respectively, or a fragment of the polypeptide. The DR3-V1 and DR3 polypeptides of the present invention shares sequence homology with human TNF RI and Fas (FIG. 4). The nucleotide sequence shown in FIG. 1 [SEQ ID NO:l] was obtained by sequencing the HTTNB61 clone, which was deposited on March 1, 1996 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given Accession Number 97456. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA).The nucleotide sequence shown in FIG. 2 [SEQ ID NO:3] was obtained by sequencing a clone obtained from a HUVEC library, which was deposited on October 10, 1996 at the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and given Accession Number _____. The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA).

Nucleic Acid Molecules

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

By "isolated" polypeptide or protein is intended a polypeptide or protein removed from its native environment. For example, recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention as are native or recombinant polypeptides which have been substantially purified by any suitable technique such as, for example, the single-step purification method disclosed in Smith and Johnson, *Gene 67*:31-40 (1988).

Using the information provided herein, such as the nucleic acid sequence set out in FIG. 1 or FIG. 2, a nucleic acid molecule of the present invention encoding a DR3-V1 or DR3 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as

starting material. Illustrative of the invention, the nucleic acid molecule described in FIG. 1 was discovered in a cDNA library derived from cells of a human testis tumor. Also illustrative of the invention, the nucleic acid molecule described in FIG. 2 was discovered in a human HUVEC cDNA library. In addition, the genes of the present invention have also been identified in cDNA libraries of the following tissues: fetal liver, fetal brain, tonsil and leukocyte. Furthermore, multiple forms of DR3 transcript are seen in Northern Blots and PCR reactions indicating that multiple variants of the transcript exists, possibly due to alternate splicing of the message.

The DR3-V1 (formerly called DDCR) gene contains an open reading frame encoding a protein of about 428 amino acid residues whose initiation codon is at position 198-200 of the nucleotide sequence shown in FIG. 1 [SEQ ID NO.1], with a leader sequence of about 35 amino acid residues, and a deduced molecular weight of about 47 kDa. Of known members of the TNF receptor family, the DR3-V1 polypeptide of the invention shares the greatest degree of homology with human TNF RI. The DR3-V1 polypeptide shown in FIG. 1 [SEQ ID NO:2] is about 20% identical and about 50% similar to human TNF RI.

The DR3 gene contains an open reading frame encoding a protein of about 417 amino acid residues whose initiation codon is at position 1-3 of the nucleotide sequence shown in FIG. 2 [SEQ ID NO:3], with a leader sequence of about 24 amino acid residues, and a deduced molecular weight of about 43 kDa. Of known members of the TNF receptor family, the DR3 polypeptide of the invention shares the greatest degree of homology with human TNF RI. The DR3 polypeptide shown in FIG. 2 [SEQ ID NO:3] is about 20% identical and about 50% similar to human TNF RI.

As indicated, the present invention also provides the mature form(s) of the DR3-V1 and DR3 protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the

same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature DR3-V1 or DR3 polypeptides having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97456 and _____, respectively, and as shown in Figure 1 (SEQ ID NO:2) and Figure 2 (SEQ ID NO:4). By the mature DR3-V1 or DR3 protein having the amino acid sequence encoded by the cDNA clones contained in the host identified as ATCC Deposit No. 97456 and _____, respectively, is meant the mature form(s) of the DR3-V1 or DR3 protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature DR3-V1 or DR3 having the amino acid sequence encoded by the cDNA clones contained in ATCC Deposit No. 97456 and _____, respectively, may or may not differ from the predicted "mature" DR3-V1 protein shown in Figure 1 (amino acids from about 36 to about 428) or DR3 protein shown in Figure 2 (amino acids from about 24 to about 417) depending on the accuracy of the predicted cleavage site based on computer analysis.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the method of McGeoch (*Virus Res. 3*:271-286 (1985)) and von Heinje (*Nucleic Acids Res. 14*:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra.* However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete DR3-V1 and DR3 polypeptides of the present invention were analyzed by a

computer program ("PSORT"). (*see* K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 35 and 36 in Figure 1 (SEQ ID NO:2) and between amino acids 24 and 25 in Figure 2 (SEQ ID NO:4). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heine. von Heinje, *supra*. Thus, the leader sequence for the DR3-V1 protein is predicted to consist of amino acid residues 1- 35 in Figure 1 (SEQ ID NO:2), while the predicted mature DR3-V1 protein consists of residues 36-428. The leader sequence for the DR3 protein is predicted to consist of amino acid residues 1- 24 in Figure 2 (SEQ ID NO:4), while the predicted mature DR3 protein consists of residues 25-417.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, as well as the variability of cleavage sites for leaders in different known proteins, the actual DR3-V1 polypeptide encoded by the deposited cDNA comprises about 428 amino acids, but may be anywhere in the range of 410-440 amino acids; and the actual leader sequence of this protein is about 35 amino acids, but may be anywhere in the range of about 25 to about 45 amino acids. The actual DR3 polypeptide encoded by the deposited cDNA comprises about 417 amino acids, but may be anywhere in the range of 400-430 amino acids; and the actual leader sequence of this protein is about 24 amino acids, but may be anywhere in the range of about 14 to about 34 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DR3-V1 DNA molecules comprising an open reading frame (ORF) shown in FIG. 1 [SEQ ID NO:1] and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 198-200 of the nucleotide sequence shown in FIG. 1 [SEQ ID NO:1] but which, due to the degeneracy of the genetic code, still encode the DR3-V1 polypeptide or a fragment thereof. Isolated nucleic acid molecules of the present invention also include DR3 DNA molecules comprising an open reading frame (ORF) shown in FIG. 2 [SEQ ID NO:3] and further include DNA molecules which comprise a sequence substantially different than all or part of the ORF whose initiation codon is at position 1-3 of the nucleotide sequence shown in FIG. 2 [SEQ ID NO:3] but which, due to the degeneracy of the genetic code, still encode the DR3 polypeptide or a fragment thereof. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the DR3-V1 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97456 on March 1, 1996. The invention provides isolated nucleic acid molecules encoding the DR3 polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No._____ on October 10, 1996. Preferably, these nucleic acid molecules will encode the

mature polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 (SEQ ID NO:1) or FIG. 2 (SEQ ID NO:3) or the nucleotide sequence of the DR3-V1 or DR3 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated DNA molecules and fragments thereof are useful as DNA probes for gene mapping by *in situ* hybridiz DR3-V1 or DR3 gene in human tissue (including testis tumor tissue) by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By fragments of an isolated DNA molecule having the nucleotide sequence shown in FIG. 1 [SEQ ID NO:1] or FIG. 2 [SEQ ID NO:3] are intended DNA fragments at least 20 bp, and more preferably at least 30 bp in length which are useful as DNA probes as discussed above. of course larger DNA fragments 50-1500 bp in length are also useful as DNA probes according to the present invention as are DNA fragments corresponding to most, if not all, of the nucleotide sequence shown in FIG. 1 [SEQ ID NO:1] or FIG. 2 [SEQ ID NO:3]. By a fragment at least 20 bp in length, for example, is intended fragments which include 20 or more bases from the nucleotide sequence in FIG. 1 [SEQ ID NO:1] or FIG. 2 [SEQ ID NO:3].

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising the DR3 extracellular domain (amino acid residues from about 36 to about 212 in FIG. 1 [SEQ ID NO:2]); a polypeptide comprising the DR3 transmembrane domain (amino acid residues from about 213 to about 235 in FIG. 1 [SEQ ID NO:2]; a polypeptide comprising the DR3 intracellular domain (amino acid residues from about 214 to about 428 in FIG. 1 [SEQ ID NO:2] ; and a polypeptide comprising the DR3 death domain (amino acid residues from about 353 to about 419 in FIG. 1 [SEQ ID NO:2]). Since the location of these domains have been predicted by computer graphics, one of ordinary skill would appreciate that the amino acid residues constituting

these domains may vary slightly (e.g., by about 1 to 15 residues) depending on the criteria used to define the domain.

Preferred nucleic acid fragments of the present invention further include nucleic acid molecules encoding epitope-bearing portions of the DR3-V1 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 1 to about 22 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 33 to about 56 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 59 to about 82 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 95 to about 112 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 122 to about 133 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 161 to about 177 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 179 to about 190 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 196 to about 205 in Figure 1 (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the DR3-V1 protein. Methods for determining other such epitope-bearing portions of the DR3-V1 protein are described in detail below.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of the DR3 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding the corresponding regions to those epitope-bearing regions of the DR3-V1 protein disclosed above. Methods for determining other such epitope-bearing portions of the DR3 protein are described in detail below.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit 97456 or ATCC Deposit _____. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50%

formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in Figure 1 (SEQ ID NO:1) or Figure 2 (SEQ ID NO:3).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the DR3-V1 cDNA shown in Figure 1 (SEQ ID NO:1)), or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

As indicated, nucleic acid molecules of the present invention which encode the DR3-V1 or DR3 polypeptide may include, but are not limited to the coding sequence for the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding a leader or secretary sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing - including splicing and polyadenylation signals,

for example - ribosome binding and stability of mRNA; additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, for instance, the polypeptide may be fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al., Proc. Natl. Acad. Sci. USA 86*: 821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson *et al., Cell 37*:767 (1984), for instance.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode for fragments, analogs or derivatives of the DR3-V1 or DR3 polypeptide. Variants may occur naturally, such as an allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions which may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions.

Further embodiments of the invention include isolated nucleic acid molecules that are at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical, to (a) a nucleotide sequence encoding the full-length DR3-V1 polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2), including the predicted leader sequence; (b) nucleotide sequence encoding the full-length DR3 polypeptide having the complete amino acid

sequence in Figure 2 (SEQ ID NO:4), including the predicted leader sequence; (c) a nucleotide sequence encoding the mature DR3-V1 polypeptide (full-length polypeptide with the leader removed) having the amino acid sequence at positions about 36 to about 428 in Figure 1 (SEQ ID NO:2); (d) a nucleotide sequence encoding the full-length DR3-V1 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97456; (e) a nucleotide sequence encoding the full-length DR3 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No._____; (f) a nucleotide sequence encoding the mature DR3-V1 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97456; (g) a nucleotide sequence encoding the mature DR3-V1 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. _____; (h) a nucleotide sequence that encodes the DR3 extracellular domain, (i) a nucleotide sequence that encodes the DR3 transmembrane domain, (j) a nucleotide sequence that encodes the DR3 intracellular domain, and (k) a nucleotide sequence that encodes the DR3 death domain; or (l) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k) above.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a DR3-V1 or DR3 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the DR3-V1 or DR3 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence

or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1, Figure 2 or to the nucleotide sequences of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3) or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having DR3 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having DR3 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having DDCR activity include, *inter alia,* (1) isolating the DR3-V1 or DR3 gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the DR3-V1 or DR3 gene, as described in Verma *et al., Human*

Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting DR3-V1 or DR3 mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1), Figure 2 (SEQ ID NO:3) or to the nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide having DR3 protein activity. By "a polypeptide having DR3 activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the DR3 protein of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay. For example, DR3 protein activity can be measured using the cell death assays performed essentially as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)) and as set forth in Example 7, below. In MCF7 cells, plasmids encoding full-length DR3 or a candidate death domain containing receptors are co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells transfected with DR3 will exhibit apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al., Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996); M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), DR3-induced apoptosis is blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk. In addition, apoptosis induced by DR3 is also blocked by dominant negative versions of FADD (FADD-DN) or FLICE (FLICE-DN/MACHa1C360S).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figure 1 (SEQ ID NO:1) or Figure 2 (SEQ ID NO:3) will

encode a polypeptide "having DR3 protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having DR3 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J.U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science 247:*1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Polynucleotide assays

This invention is also related to the use of the DR3-V1 or DR3 polynucleotides to detect complementary polynucleotides such as, for example, as a diagnostic reagent. Detection of a mutated form of DR3-V1 or DR3 associated with a dysfunction will provide a diagnostic tool that can add or define a diagnosis of a disease or susceptibility to a disease which results from under-expression over-expression or altered expression of DR3-V1 or DR3 or a soluble form thereof, such as, for example, tumors or autoimmune disease.

Individuals carrying mutations in the DR3-V1 or DR3 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. (Saiki *et al., Nature 324:*163-166 (1986)). RNA or cDNA may also be used in the same ways. As an example, PCR primers complementary to the nucleic acid

encoding DR3-V1 or DR3 can be used to identify and analyze DR3-V1 or DR3 expression and mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled DR3-V1 or DR3 RNA or alternatively, radiolabeled DR3-V1 or DR3 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between a reference gene and genes having mutations also may be revealed by direct DNA sequencing. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of such methods can be greatly enhanced by appropriate use of PCR or another amplification method. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al., Science 230*:1242 (1985)).

Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al., Proc. Natl. Acad. Sci. USA 85*: 4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA

sequencing or the use of restriction enzymes, (e.g., restriction fragment length polymorphisms ("RFLP") and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations also can be detected by *in situ* analysis.

Chromosome assays

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a DR3-V1 or a DR3 gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA the is used for *in situ* chromosome mapping using well known techniques for this purpose.

In addition, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA as short as 50 or 60. For a review of this technique, see Verma *et al.*, *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian*

Inheritance in Man, available on line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes)).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Vectors and Host Cells

The present invention also relates to vectors which include DNA molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells can be genetically engineered to incorporate nucleic acid molecules and express polypeptides of the present invention. The polynucleotides may be introduced alone or with other polynucleotides. Such other polynucleotides may be introduced independently, co-introduced or introduced joined to the polynucleotides of the invention.

In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells as polynucleotides, preferably DNA, by well known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case viral propagation generally will occur only in complementing host cells.

Preferred among vectors, in certain respects, are those for expression of polynucleotides and polypeptides of the present invention. Generally, such vectors comprise cis-acting control regions effective for expression in a host operatively linked to the polynucleotide to be expressed. Appropriate trans-

acting factors either are supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

A great variety of expression vectors can be used to express a polypeptide of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used for expression in this regard.

The DNA sequence in the expression vector is operatively linked to appropriate expression control sequence(s)), including, for instance, a promoter to direct mRNA transcription. Representatives of such promoters include the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name just a few of the well-known promoters. In general, expression constructs will contain sites for transcription, initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

In addition, the constructs may contain control regions that regulate as well as engender expression. Generally, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

Vectors for propagation and expression generally will include selectable markers. Such markers also may be suitable for amplification or the vectors may contain additional markers for this purpose. In this regard, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic

trait for selection of transformed host cells. Preferred markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing *E. coli* and other bacteria.

The vector containing the appropriate DNA sequence as described elsewhere herein, as well as an appropriate promoter, and other appropriate control sequences, may be introduced into an appropriate host using a variety of well known techniques suitable to expression therein of a desired polypeptide. Representative examples of appropriate hosts include bacterial cells, such as *E. coli*, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Hosts for of a great variety of expression constructs are well known, and those of skill will be enabled by the present disclosure readily to select a host for expressing a polypeptides in accordance with this aspect of the present invention.

Among vectors preferred for use in bacteria are pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. These vectors are listed solely by way of illustration of the many commercially available and well known vectors available to those of skill in the art.

Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host and expression in the host are routine skills in the art.

The present invention also relates to host cells containing the above-described constructs discussed above. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al., Basic Methods in Molecular Biology* (1986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, region also may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett *et al., Journal of Molecular Recognition*, Vol. 8:52-58 (1995) and K. Johanson *et al., The Journal of Biological Chemistry*, Vol. 270, No. 16:9459-9471 (1995).

The DR3 and DR3-V1 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

DR3-V1 or DR3 polynucleotides and polypeptides may be used in accordance with the present invention for a variety of applications, particularly those that make use of the chemical and biological properties of DR3. Among these are applications in treatment of tumors, resistance to parasites, bacteria and viruses, to induce proliferation of T-cells, endothelial cells and certain hematopoietic cells, to treat restenosis, graft vs. host disease, to regulate anti-viral responses and to prevent certain autoimmune diseases after stimulation of DR3 by an agonist. Additional applications relate to diagnosis and to treatment of disorders of cells, tissues and organisms. These aspects of the invention are discussed further below.

DR3 Polypeptides and Fragments

The invention further provides an isolated DR3-V1 or DR3 polypeptide having the amino acid sequence shown in FIG. 1 [SEQ ID NO:2] and FIG. 2[SEQ ID NO:4], respectively, or a fragment thereof. It will be recognized in the art that some amino acid sequence of DR3-V1 or DR3 can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

Thus, the invention further includes variations of the DR3-V1 or DR3 protein which show substantial DR3 protein activity or which include regions of DR3-V1 or DR3 such as the protein fragments discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U. *et al., Science 247:*1306-1310 (1990).

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the DR3-V1 or DR3 protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol. 2*:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-α to only one of the two known types of TNF receptors. Thus, the DR3-V1 or DR3 receptor of the present

invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Amino acids in the DR3-V1 or DR3 protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization,

nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of the DR3-V1 or DR3 polypeptide is substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988).

The polypeptides of the present invention also include the polypeptide encoded by the deposited cDNA including the leader, the mature polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein), the polypeptide of Figure 1 (SEQ ID NO:2) or Figure 2 (SEQ ID NO:4) including the leader, the polypeptide of Figure 1 (SEQ ID NO:2) or Figure 2 (SEQ ID NO:4) minus the leader, the extracellular domain, the transmembrane domain, the intracellular domain, soluble polypeptides comprising all or part of the extracellular and intracelluar domains but lacking the transmembrane domain as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptide encoded by the deposited cDNA clones, to the polypeptide of Figure 1 (SEQ ID NO:2) or Figure 2 (SEQ ID NO:4), and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a DR3-V1 or DR3 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the DR3-V1 or DR3 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the

reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 1 (SEQ ID NO:2), or Figure 2 (SEQ ID NO:4) or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The present inventors have discovered that the DR3-V1 polypeptide is a 428 residue protein exhibiting three main structural domains. First, the ligand binding domain was identified within residues from about 36 to about 212 in FIG. 1 [SEQ ID NO:2]. Second, the transmembrane domain was identified within residues from about 213 to about 235 in FIG. 1 [SEQ ID NO:2]. Third, the intracellular domain was identified within residues from about 236 to about 428 in FIG. 1 [SEQ ID NO:2]. Importantly, the intracellular domain includes a death domain at residues from about 353 to about 419. Further preferred fragments of the polypeptide shown in FIG. 1 [SEQ ID NO:2] include the mature protein from residues about 36 to about 428 and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain.

The present inventors have also discovered that the DR3 polypeptide is a 417 residue protein exhibiting three main structural domains. First, the ligand

binding domain was identified within residues from about 25 to about 201 in FIG. 2 [SEQ ID NO:4]. Second, the transmembrane domain was identified within residues from about 202 to about 224 in FIG. 2 [SEQ ID NO:4]. Third, the intracellular domain was identified within residues from about 225 to about 417 in FIG. 2 [SEQ ID NO:4]. Importantly, the intracellular domain includes a death domain at residues from about 342 to about 408. Further preferred fragments of the polypeptide shown in FIG. 2 [SEQ ID NO:4] include the mature protein from residues about 25 to about 417 and soluble polypeptides comprising all or part of the extracellular and intracellular domains but lacking the transmembrane domain. As one of skill in the art will recognize, the full length polypeptides encoded by the DR3-V1 and DR3 cDNA differ only in the amino acid sequence of the leader peptide. The first 24 amino acids of the polypeptide shown in Figure 1 are replaced by the first 13 amino acids shown in Figure 2 but the rest of the amino acid sequence is the same. Thus, both the DR3-V1 cDNA and DR3 cDNA encode an identical mature protein having the same biological activity.

Thus, the invention further provides DR3-V1 or DR3 polypeptides encoded by the deposited cDNA clones including the leader and DR3-V1 or DR3 polypeptide fragments selected from the mature protein, the extracellular domain, the transmembrane domain, the intracellular domain, and the death domain.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide described herein. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al., Proc. Natl. Acad. Sci. USA 81*:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al., Cell 37*:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate DR3-specific antibodies include: a polypeptide comprising amino acid residues from about 1 to about 22 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 33 to about 56 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 59 to about 82 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 95 to about 112 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 122 to about 133 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 161 to about 177 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 179 to about 190 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 196 to about 205 in

Figure 1 (SEQ ID NO:2). In additon, antigenic polypeptides or peptides include polypeptides comprising the amino acid residues that are the corresponding residues to those polypeptides of DR3-V1 disclosed above. As indicated above, the inventors have determined that the above polypeptide fragments are antigenic regions of the DR3-V1 and DR3 protein.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R.A., "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids," *Proc. Natl. Acad. Sci. USA 82*:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate,DR3-V1 or DR3 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84-86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric DR3-V1 or DR3 protein or protein fragment alone (Fountoulakis *et al., J Biochem 270*:3958-3964 (1995)).

Polypeptide assays

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of DR3-V1 or DR3 protein, or the soluble form thereof, in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the

invention for detecting over-expression of DR3-V1 or DR3, or soluble form thereof, compared to normal control tissue samples may be used to detect the presence of tumors, for example. Assay techniques that can be used to determine levels of a protein, such as an DR3 protein of the present invention, or a soluble form thereof, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Assaying DR3-V1 or DR3 protein levels in a biological sample can occur using any art-known method. Preferred for assaying DR3-V1 or DR3 protein levels in a biological sample are antibody-based techniques. For example,DR3-V1 or DR3 protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., *et al., J. Cell. Biol. 101:*976-985 (1985); Jalkanen, M., *et al., J. Cell . Biol. 105:*3087-3096 (1987)).

Other antibody-based methods useful for detecting DR3-V1 or DR3 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as glucose oxidase, radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulphur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Therapeutics

The Tumor Necrosis Factor (TNF) family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (Goeddel, D.V. *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol. 51*:597-609 (1986), Cold Spring Harbor; Beutler, B., and Cerami, A., *Annu. Rev. Biochem. 57*:505-518 (1988); Old, L.J., *Sci. Am. 258*:59-75 (1988); Fiers, W., *FEBS Lett. 285*:199-224 (1991)). The TNF-family ligands induce such various

cellular responses by binding to TNF-family receptors, including the DR3-V1 or DR3 of the present invention. Cells which express the DR3-V1 or DR3 polypeptide and are believed to have a potent cellular response to DR3-V1 or DR3 ligands include lymphocytes, fibroblasts, macrophages, synovial cells, activated T-cells, lymphoblasts and epithelial cells. By "a cellular response to a TNF-family ligand" is intended any genotypic, phenotypic, and/or morphologic change to a cell, cell line, tissue, tissue culture or patient that is induced by a TNF-family ligand. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death-is a physiological mechanism involved in the deletion of peripheral T lymphocytes of the immune system, and its dysregulation can lead to a number of different pathogenic processes (Ameisen, J.C., *AXDS 8*:1197-1213 (1994); Krammer, P.H. *et al., Curr. Opin. Immunol. 6*:279-289 (1994)).

Diseases associated with increased cell survival, or the inhibition of apoptosis, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, such as breast cancer, prostrate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), information graft v. host disease, acute graft rejection, and chronic graft rejection. Diseases associated with increased apoptosis include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Thus, in one aspect, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the DR3-V1 or DR3 polypeptide an

effective amount of DR3-V1 or DR3 ligand, analog or an agonist capable of increasing DR3-V1 or DR3 mediated signaling. Preferably, DR3-V1 or DR3 mediated signaling is increased to treat a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited. An agonist can include soluble forms of DR3-V1 or DR3 and monoclonal antibodies directed against the DR3-V1 or DR3 polypeptide.

In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the, DR3-V1 or DR3 polypeptide an effective amount of an antagonist capable of decreasing DR3-V1 or DR3 mediated signaling. Preferably, DR3-V1 or DR3 mediated signaling is decreased to treat a disease wherein increased apoptosis or NFkB expression is exhibited. An antagonist can include soluble forms of DR3-V1 or DR3 and monoclonal antibodies directed against the DR3-V1 or DR3 polypeptide.

By "agonist" is intended naturally occurring and synthetic compounds capable of enhancing or potentiating apoptosis. By "antagonist" is intended naturally occurring and synthetic compounds capable of inhibiting apoptosis. Whether any candidate "agonist" or "antagonist" of the present invention can enhance or inhibit apoptosis can be determined using art-known TNF-family ligand/receptor cellular response assays, including those described in more detail below.

One such screening procedure involves the use of melanophores which are transfected to express the receptor of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed, for example, for screening for a compound which inhibits (or enhances) activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both a TNF-family ligand and the candidate antagonist (or agonist). Inhibition or enhancement of the signal generated by the ligand indicates that the compound is an antagonist or agonist of the ligand/receptor signaling pathway.

Other screening techniques include the use of cells which express the receptor (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science 246*:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g., signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the receptor into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing in cells a construct wherein the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the receptor such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

Further screening assays for agonist and antagonist of the present invention are described in Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267(7)*:4304-4307(1992).

Thus, in a further aspect, a screening method is provided for determining whether a candidate agonist or antagonist is capable of enhancing or inhibiting a cellular response to a TNF-family ligand. The method involves contacting cells which express the DR3-V1 or DR3 polypeptide with a candidate compound and a TNF-family ligand, assaying a cellular response, and comparing the cellular response to a standard cellular response, the standard being assayed when contact is made with the ligand in absence of the candidate compound, whereby an increased cellular response over the standard indicates that the candidate compound is an agonist of the ligand/receptor signaling pathway and a decreased cellular response compared to the standard indicates that the candidate compound is an antagonist of the ligand/receptor signaling pathway. By "assaying a cellular response" is intended qualitatively or quantitatively measuring a cellular response to a candidate compound and/or a TNF-family ligand (e.g., determining or estimating an increase or decrease in T cell proliferation or tritiated thymidine labeling). By the invention, a cell expressing the DR3-V1 or DR3 polypeptide can be contacted with either an endogenous or exogenously administered TNF-family ligand.

Agonist according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor β, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonist include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and β-amyloid peptide. (*Science 267*:1457-1458 (1995)). Further preferred agonist include polyclonal and monoclonal antibodies raised against the DR3-V1 or DR3 polypeptide, or a fragment thereof. Such agonist antibodies raised against a TNF-family receptor are disclosed in Tartaglia, L.A., *et al., Proc. Natl. Acad. Sci.*

USA 88:9292-9296 (1991); and Tartaglia, L.A., and Goeddel, D.V., *J. Biol. Chem. 267* (7):4304-4307 (1992) See, also, PCT Application WO 94/09137.

Antagonist according to the present invention include naturally occurring and synthetic compounds such as, for example, the CD40 ligand, neutral amino acids, zinc, estrogen, androgens, viral genes (such as Adenovirus *E1B*, Baculovirus *p35* and *IAP*, Cowpox virus *crmA*, Epstein-Barr virus *BHRF1*, *LMP-1*, African swine fever virus *LMW5-HL*, and Herpesvirus yl 34.5), calpain inhibitors, cysteine protease inhibitors, and tumor promoters (such as PMA, Phenobarbital, and α-Hexachlorocyclohexane).

Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, *J. Neurochem. 56*:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee *et al., Nucleic Acids Research 6*:3073 (1979); Cooney *et al., Science 241*:456 (1988); and Dervan *et al., Science 251*:1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into receptor polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the receptor.

Further antagonist according to the present invention include soluble forms of DR3-V1 or DR3, i.e.,DR3-V1 or DR3 fragments that include the ligand binding domain from the extracellular region of the full length receptor. Such

soluble forms of the receptor, which may be naturally occurring or synthetic, antagonize DR3-V1 or DR3 mediated signaling by competing with the cell surface DR3-V1 or DR3 for binding to TNF-family ligands. Thus, soluble forms of the receptor that include the ligand binding domain are novel cytokines capable of inhibiting apoptosis induced by TNF-family ligands. These are preferably expressed as dimers or trimers, since these have been shown to be superior to monomeric forms of soluble receptor as antagonists, e.g., IgGFc-TNF receptor family fusions. Other such cytokines are known in the art and include Fas B (a soluble form of the mouse Fas receptor) that acts physiologically to limit apoptosis induced by Fas ligand (Hughes, D.P. and Crispe, I.N., *J. Exp. Med. 182*:1395-1401 (1995)).

The experiments set forth in Examples 6 and 7 demonstrate that DR3 is a death domain-containing molecule capable of triggering both apoptosis and NF-kB activation, two pathways dominant in the regulation of the immune system. The experiments also demonstrate the internal signal transduction machinery of this novel cell death receptor. In addition, the experiments set forth below demonstrate that DR3-induced apoptosis was blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk. Importantly, apoptosis induced by DR3 was also blocked by dominant negative versions of FADD (FADD-DN) or FLICE (FLICE-DN/MACHa1C360S), which were previously shown to inhibit death signaling by Fas/APO-1 and TNFR-1. Thus, inhibitors of ICE-like proteases, FADD-DN and FLICE-DN/MACHa1C360S could also be used as antagonists for DR3 activity.

The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and $F(ab')_2$ fragments) which are capable of binding an antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al., J. Nucl. Med. 24*:316-325 (1983)).

Antibodies according to the present invention may be prepared by any of a variety of methods using DR3-V1 or DR3 immunogens of the present

invention. As indicated, such DR3-V1 or DR3 immunogens include the full length DR3-V1 or DR3 polypeptide (which may or may not include the leader sequence) and DR3-V1 or DR3 polypeptide fragments such as the ligand binding domain, the transmembrane domain, the intracellular domain and the death domain.

Proteins and other compounds which bind the DR3-V1 or DR3 domains are also candidate agonist and antagonist according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature 340*:245-246 (1989)). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, J. *et al., Cell 75*:791-803 (1993); Zervos, A.S. *et al., Cell 72*:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to either the DR3-V1 or DR3 ligand binding domain or to the DR3-V1 or DR3 intracellular domain. Such compounds are good candidate agonist and antagonist of the present invention.

By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, the DR3-V1 or DR3 ligand, TNF-α, lymphdtoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40, CD27, CD30, 4-lBB, OX40 and nerve growth factor (NGF).

Representative therapeutic applications of the present invention are discussed in-more detail below. The state of immunodeficiency that defines AIDS is secondary to a decrease in the number and function of $CD4^+$ T-lymphocytes. Recent reports estimate the daily loss of $CD4^+$ T cells to be between 3.5×10^7 and 2×10^9 cells (Wei X., *et al., Nature 373*:117-122 (1995)). One cause of $CD4^+$ T cell depletion in the setting of HIV infection is believed to be HIV-induced apoptosis. Indeed, HIV-induced apoptotic cell death has been demonstrated not only *in vitro* but also, more importantly, in infected individuals (Ameisen, J.C., *AIDS 8*:1197-1213 (1994) ; Finkel, T.H., and Banda, N.K., *Curr.*

Opin. Immunol. 6:605-615(1995); Muro-Cacho, C.A. *et al., J. Immunol. 154*:5555-5566 (1995)). Furthermore, apoptosis and $CD4^+$ T-lymphocyte depletion is tightly correlated in different animal models of AIDS (Brunner, T., *et al., Nature 373*:441-444 (1995); Gougeon, M.L., *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)) and, apoptosis is not observed in those animal models in which viral replication does not result in AIDS (Gougeon, M.L. *et al., AIDS Res. Hum. Retroviruses 9*:553-563 (1993)). Further data indicates that uninfected but primed or activated T lymphocytes from HIV-infected individuals undergo apoptosis after encountering the TNF-family ligand FasL. Using monocytic cell lines that result in death following HIV infection, it has been demonstrated that infection of U937 cells with HIV results in the *de novo* expression of FasL and that FasL mediates HIV-induced apoptosis (Badley, A.D. *et al., J. Virol. 70*:199-206 (1996)). Further the TNF-family ligand was detectable in uninfected macrophages and its expression was upregulated following HIV infection resulting in selective killing of uninfected CD4 T-lymphocytes (Badley, A.D *et al., J. Virol. 70*:199-206 (1996)). Thus, by the invention, a method for treating HIV^+ individuals is provided which involves administering an antagonist of the present invention to reduce selective killing of CD4 T-lymphocytes. Modes of administration and dosages are discussed in detail below.

In rejection of an allograft, the immune system of the recipient animal has not previously been primed to respond because the immune system for the most part is only primed by environmental antigens. Tissues from other members of the same species have not been presented in the same way that, for example, viruses and bacteria have been presented. In the case of allograft rejection, immunosuppressive regimens are designed to prevent the immune system from reaching the effector stage. However, the immune profile of xenograft rejection may resemble disease recurrence more that allograft rejection. In the case of disease recurrence, the immune system has already been activated, as evidenced by destruction of the native islet cells. Therefore, in disease recurrence the immune system is already at the effector stage. Agonist of the present invention

are able to suppress the immune response to both allografts and xenografts because lymphocytes activated and differentiated into effector cells will express the DR3-V1 or DR3 polypeptide, and thereby are susceptible to compounds which enhance apoptosis. Thus, the present invention further provides a method for creating immune privileged tissues. Antagonist of the invention can further be used in the treatment of Inflammatory Bowel-Disease.

DR3, like TNFR1, also activates the NF-kB transcription factor, which is very closely associated with the stimulation of cytokine (e.g., IL-8) and adhesion molecule (e.g., ELAM) transcription. Hence, like TNF, the ligand (or agonist) for DR3 and DR3-V1 may in some circumstances be proinflammatory, and antagonists may be useful reagents for blocking this response. Thus, DR3 and DR3-V1 antagonists may be useful for treating inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

In addition, due to lymphoblast expression of DR3, soluble DR3, agonist or antagonist mABs may be used to treat this form of cancer. Further, soluble DR3 or neutralizing mABs may be used to treat various chronic and acute forms of inflammation such as rheumatoid arthritis, osteoarthritis, psoriasis, septicemia, and inflammatory bowel disease.

Modes of Administration

The agonist or antagonists described herein can be administered *in vitro*, *ex vivo*, or *in vivo* to cells which express the receptor of the present invention. By administration of an "effective amount" of an agonist or antagonist is intended an amount of the compound that is sufficient to enhance or inhibit a cellular response to a TNF-family ligand and include polypeptides. In particular, by administration of an "effective amount" of an agonist or antagonists is intended an amount effective to enhance or inhibit DR3-V1 or DR3 mediated apoptosis. Of course, where apoptosis is to be enhanced, an agonist according to the present invention can be co-administered with a TNF-family ligand. One of ordinary

skill will appreciate that effective amounts of an agonist or antagonist can be determined empirically and may be employed in pure form or in pharmaceutically acceptable salt, ester or prodrug form. The agonist or antagonist may be administered in compositions in combination with one or more pharmaceutically acceptable excipients.

It will be understood that, when administered to a human patient, the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will depend upon factors well known in the medical arts.

As a general proposition, the total pharmaceutically effective amount of DDCR polypeptide administered parenterally per dose will be in the range of about 1 μg/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the DDCR agonists or antagonists is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Dosaging may also be arranged in a patient specific manner to provide a predetermined concentration of an agonist or antagonist in the blood, as determined by the RIA technique. Thus patient dosaging may be adjusted to achieve regular on-going trough blood levels, as measured by RIA, on the order of from 50 to 1000 ng/ml, preferably 150 to 500 ng/ml.

Pharmaceutical compositions are provided comprising an agonist or antagonist and a pharmaceutically acceptable carrier or excipient, which may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. Importantly, by co-administering an agonist and a TNF-family ligand, clinical side effects can be reduced by using

lower doses of both the ligand and the agonist. It will be understood that the agonist can be "co-administered" either before, after, or simultaneously with the TNF-family ligand, depending on the exigencies of a particular therapeutic application. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Pharmaceutical compositions of the present invention for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use.

In addition to soluble DR3-V1 or DR3 polypeptides, DR3-V1 or DR3 polypeptide containing the transmembrane region can also be used when appropriately solubilized by including detergents, such as CHAPS or NP-40, with buffer.

Example 1

Expression and Purification in E. coll

The DNA sequence encoding the mature DR3-V1 protein in the deposited cDNA clone (ATCC No. 97456) is amplified using PCR oligonucleotide primers specific to the amino terminal sequences of the DR3-V1 protein and to vector sequences 3′ to the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5′ and 3′ sequences respectively.

The following primers are used for expression of DR3 extracellular domain in *E. coli* 5' primer 5'-GCGCCATGGGGGCCCGGCGGCAG-3' (SEQ ID NO:7) contains an NcoI site and 15 nucleotide starting from 290 nucleotide to 304 FIG. 1. 3' primer 5'-GCGAAGCTTCTAGGACCCAGAACATCTGCC-3'

complimentary to nucleotide from 822 to 840 in FIG. 1. Vector is pQE60. The protein is not tagged.

The restriction sites are convenient to restriction enzyme sites in the bacterial expression vector pQE60, which are used for bacterial expression in these examples. (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Amp^r") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS").

The amplified DR3-V1 DNA and the vector pQE60 both are digested with Nco I and HindIII and the digested DNAs are then ligated together. Insertion of the DDCR protein DNA into the restricted pQE60 vector places the DR3-V1 protein coding region downstream of and operably linked to the vector's IPTG-inducible promoter and in-frame with an initiating AUG appropriately positioned for translation of DR3-V1 protein.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing DR3-V1 protein, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μg/ml) and kanamycin (25 μg/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside

("IPTG") is then added to a final concentration of 1 mM to induce transcription from *lac* repressor sensitive promoters, by inactivating the *lac*I repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation and disrupted, by standard methods. Inclusion bodies are purified from the disrupted cells using routine collection techniques, and protein is solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate-buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 μ/ml.

Example 2

Expression in Mammalian Cells

Most of the vectors used for the transient expression of a given gene sequence in mammalian cells carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g. COS cells) which express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g. RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, also cellular signals can be used (e.g. human actin, promoter). Suitable expression vectors for use in practicing the present invention include, for

pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC67109). Mammalian host cells that could be used include, human Hela, 283, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1 African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells such as

Alternatively, a gene of interest can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Using this marker, the mammalian cells are grown in increasing amounts of methotrexate for selection and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pCl and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al., Molecular and Cellular Biology* 438:44701 (March 1985)), plus a fragment of the CMV-enhancer (Boshart *et al., Cell 41*:521-530 (1985)). Multiple cloning sites, e.g. with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 2A

Expression of extracellular soluble domain of DR3-V1 and DR3 in COS cells

The expression plasmid, pDR3-V1 HA, is made by cloning a cDNA encoding DR3-V1 (ATCC No. 97456) into the expression vector pcDNAI/Amp

is made by cloning a cDNA encoding DR3 (ATCC No. _____) into the expression vector pcDNAI/Amp

The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cell; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

A DNA fragment encoding the entire DR3-V1 or Dr3 precursor and a HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al., Cell 37*:767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is as follows:

The DR3-V1 or DR3 cDNA of the deposit clone is amplified using primers that contained convenient restriction sites, much as described above regarding the construction of expression vectors for expression of DR3-V1 or DR3 in *E. coli* and *S. fugiperda.*

To facilitate detection, purification and characterization of the expressed DR3-V1 or DR3, one of the primers contains a hemagglutinin tag ("HA tag") as described above.

Suitable primers for DR3-V1 include the following, which are used in this example, the 5' primer, 5' CGCGGATCCGCCATCATGGAGGAGACGCAGCAG 3' (SEQ ID NO:9) contains the underlined BamHI site, an ATG start codon and 5 codons thereafter.

Suitable primers for DR3 include the following, which are used in this example, the 5' primer, 5'

CGCGGATCCGCCATCATGGAGCAGCGGCCGCGG 3' (SEQ ID NO:10) contains the underlined BamHI site, an ATG start codon and 5 codons thereafter.

The 3' primer for both DR3 and DR3-V1, containing the underlined XbaI site, stop codon, hemagglutinin tag and last 14 nucleotide of 3' coding sequence (at the 3' end) has the following sequence:

5'GCGTCTAGATCAAAGCGTAGTCTGGGACGTCGTATGGGTACGGGC CGCGCTGCA 3' (SEQ ID NO:11).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and XbaI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the DR3-V1 or DR3-encoding fragment.

For expression of recombinant DR3-V1 or DR3, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al., Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, NY (1989).

Cells are incubated under conditions for expression of DR3-V1 or DR3 by the vector.

Expression of the DR3-V1 HA fusion protein or the DR3 HA fusion protein is detected by radiolabelling and immunoprecipitation, using methods described in, for example Harlow *et al., Antibodies: a Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing ^{35}S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and then lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell

The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 2B

Expression and purification of human DR3-V1 and DR3 using the CHO Expression System

The vector pC1 is used for the expression of DR3-V1 or DR3 (ATCC No. 97456 or ATCC No. _____, respectively) protein. Plasmid pC1 is a derivative of the plasmid pSV2-dhfr [ATCC Accession No. 37146]. Both plasmids contain the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F.W., Kellems, R.M., Bertino, J.R., and Schimke, R.T., 1978, J. Biol. Chem. 253:1357-1370, Hamlin, J.L. and Ma, C. 1990, Biochem. et Biophys. Acta, 1097:107-143, Page, M.J. and Sydenham, M.A. 1991, Biotechnology Vol. 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene it is usually co-amplified and over-expressed. It is state of the art to develop cell lines carrying more than 1,000 copies of the genes. Subsequently, when the methotrexate is withdrawn, cell lines contain the amplified gene integrated into the chromosome(s).

Plasmid pC1 contains for the expression of the gene of interest a strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, *et al.*, Molecular and Cellular Biology, March 1985:438-4470) plus a fragment

cytomegalovirus (CMV) (Boshart *et al.*, *Cell 41*:521-530, 1985). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI followed by the 3′ intron and the polyadenylation site of the rat preproinsulin gene. Other high efficient promoters can also be used for the expression, e.g., the human β-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well.

Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC1 is digested with the restriction enzyme BamHI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding DR3-V1 or DR3 in the deposited cDNA clones are amplified using PCR oligonucleotide primers specific to the amino acid carboxyl terminal sequence of the DR3-V1 or DR3 protein and to vector sequences 3' to the gene. Additional nucleotides containing restriction sites to facilitate cloning are added to the 5' and 3' sequences respectively.

The 5' oligonucleotide primer for DR3-V1 has the sequence 5' CGCGGATCCGCCATCATGGAGGAGACGCAGCAG 3' (SEQ ID NO:12) containing the underlined BamHI restriction site, which encodes a start AUG, followed by the Kozak sequence ad 18 nucleotides of the DR3-V1 coding sequence set out in FIG. 1 beginning with the lst base of the ATG codon.

The 5' oligonucleotide primer for DR3 has the sequence 5' CGCGGATCCGCCATCATGGAGCAGCGGCCGCGG 3' (SEQ ID NO:13) containing the underlined BamHI restriction site, which encodes a start AUG, followed by the Kozak sequence ad 18 nucleotides of the DR3 coding sequence set out in FIG. 2 beginning with the lst base of the ATG codon.

The 3' primer for both DR3 and DR3-V1 has the sequence 5' CGCGGATCCTCACGGGCCGCGCTGCA 3' (SEQ ID NO:14) containing the underlined BamHI restriction site followed by 17 nucleotides complementary to the last 14 nucleotides of the DR3-V1 or DR3 coding sequence set out in FIG. 1 or FIG. 2, respectively, plus the stop codon.

The restrictions sites are convenient to restriction enzyme sites in the CHO expression vectors pC1.

The amplified DR3 or DR3-V1 DNA and the vector pC1 both are digested with BamHI and the digested DNAs then ligated together. Insertion of the DR3-V1 or DR3 DNA into the BamHI restricted vector placed the DR3-V1 or DR3 coding region downstream of and operably linked to the vector's promoter. The sequence of the inserted gene is confirmed by DNA sequencing.

Transfection of CHO-DHFR-cells

Chinese hamster ovary cells lacking an active DHFR enzyme are used for transfection. 5 μg of the expression plasmid C1 are cotransfected with 0.5 μg of the plasmid pSVneo using the lipofecting method (Felgner *et al.*, *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the gene neo from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) and cultivated from 10-14 days. After this period, single clones are trypsinized and then seeded in 6-well petri dishes using different concentrations of methotrexate (25 nM, 50 nM, 100 nM, 200 nM, 400 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (500 nM, 1 μM, 2 μM, 5 μM). The same procedure is repeated until clones grow at a concentration of 100 μM.

The expression of the desired gene product is analyzed by Western blot analysis and SDS-PAGE.

Example 3

Cloning and expression of the soluble extracellular domain of DR3-V1 and DR3 in a baculovirus expression system

The cDNA sequence encoding the soluble extracellular domain of DR3-V1 or DR3 protein in the deposited clone (ATCC No. 97456 or ATCC No. _____, respectively) is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer for DR3-V1 has the sequence 5' CGCGGATCC GCCATCATGGAGGAGACGCAGCAG 3' (SEQ ID NO:15) containing the underlined BamHI restriction enzyme site followed by Kozak sequence and a number of bases of the sequence of DR3-V1 of FIG. 1. Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding DR3-V1 provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987) is appropriately located in the vector portion of the construct.

The 5' primer for DR3 has the sequence 5' CGCGGATCC GCCATCATGGAGCAGCGGCCGCGG 3' (SEQ ID NO:16) containing the underlined BamHI restriction enzyme site followed by Kozak sequence and a number of bases of the sequence of DR3 of FIG. 2. Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding DR3 provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987) is appropriately located in the vector portion of the construct.

The 3' primer for both DR3 and DR3-V1 has the sequence 5' GCGAGATCTAGTCTGGACCC AGAACATCTGCCTCC 3' (SEQ ID NO:17) containing the underlined XbaI restriction followed by nucleotides complementary to the DR3-V1 or DR3 nucleotide sequence set out in FIG. 1 or FIG.2, respectively, followed by the stop codon.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean, " BIO 101 Inc., La Jolla, Ca.) The fragment then is digested with BamHI and Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein F2.

The vector pA2 is used to express the DR3-V1 or DR3 protein in the baculovirus expression system, using standard methods, such as those described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedron promoter of the Autograph californica nuclear polyhedrosis virus (ACMNPV) followed by convenient restriction sites. For an easy selection of recombinant virus the beta-galactosidase gene from *E. coli* is inserted in the same orientation as the polyhedron promoter and is followed by the polyadenylation signal of the polyhedron gene. The polyhedron sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pA2, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described in Luckow *et al., Virology 170*:31-39, among others.

The plasmid is digested with the restriction enzymes Bam HI and XbaI and then is dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V2".

Fragment F2 and the dephosphorylated plasmid V2 are ligated together with T4 DNA ligase. *E. coli* HB101 cells are transformed with ligation mix and spread on culture plates. Bacteria are identified that contain the plasmid with the human DDCR gene by digesting DNA from individual colonies using BamHI and

XbaI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac DR3-V1 or pBac DR3.

5 µg of the plasmid pBac DR3-V1 or pBac DR3 is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner *et al., Proc. Natl. Acad. Sci. USA 84*:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pBac DR3-V1 are mixed in a sterile well of a microliter plate containing 50 µl of serum free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, cited above. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10).

Four days after serial dilution, the virus is added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses is then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four

days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. A clone containing properly inserted DR3-V1 or DR3 is identified by DNA analysis including restriction mapping and sequencing. This is designated herein as V- DR3-V1 or V-DR3.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-DR3-V1 at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Gaithersburg). 42 hours later, 5 gCi of ^{35}S-methionine and 5 μCi ^{35}S cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

Example 4

A. Tissue distribution of DR3-V1 gene expression

Northern blot analysis is carried out to examine DR3-V1 gene (ATCC No. 97456) expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the DR3-V1 protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for DR3-V1 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following

hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures Expression of DR3-V1 was detected in tissues enriched in lymphocytes including peripheral blood leukocytes (PBLs), thymus, spleen, colon, and small intestine. DR3-V1 expression appears to be restricted to lymphocyte compartments, it can be envisaged that DR3-V1 plays a role in lymphocyte homeostasis.

B. Tissue distribution of DR3 gene expression

Northern blot analysis is carried out to examine DR3 gene (ATCC No. _____) expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the DR3 protein (SEQ ID NO:1) is labeled with ^{32}P using the *redi*prime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for DR3 mRNA.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

Expression of DR3 was detected in tissues enriched in lymphocytes including peripheral blood leukocytes (PBLs), thymus, spleen, colon, and small intestine. By contrast, TNFR-1 is ubiquitously expressed and Fas/APO-1 is expressed in lymphocytes, liver, heart, lung, kidney, and ovary (Watanabae-Fukunaga, *et al., J. Immunol 148:*1274-9 (1992)).

DR3 expression appears to be restricted to lymphocyte compartments, it can be envisaged that DR3 plays a role in lymphocyte homeostasis.

C. *Northern Blot analysis of DR3 in various cell lines*

Methods

Cells

Unless stated otherwise, cell lines were obtained from the American Type Culture Collection (Rockville, MD). The myeloid (Koeffler *et al.* (1980); Koeffler (1983); Harris and Ralph (1985); and Tucker *et al.* (1987) and B-cell lines (Jonak *et al.* (1922)) studied represent cell types at different stages of the differentiation pathway. KG1a and PLB 985 cells (Tucker *et al.* (1987)) were obtained from H.P. Koeffler (UCLA School of Medicine). BJA-B was from Z. Jonak (SmithKline Beecham). TF274, a stromal cell line exhibiting osteoblastic features, was generated from the bone marrow of a healthy male donor (Z. Jonak and K.B. Tan, unpublished). Primary carotid artery endothelial cells were purchased from Clonetics Corp. (San Diego, CA) and monocytes were prepared by differential centrifugation of peripheral blood mononuclear cells and adhesion to tissue culture dish. CD19+, CD4+ and CD8+ cells (>90% pure) were isolated with cell type specific immunomagnetic beads (Drynal, Lake Success, NY).

RNA Analysis

Total RNA of adult tissues were purchased from Clonetech (Palo Alto, CA). Total RNA was extracted from cell lines (in exponential growth phase) and primary cells with TriReagent (Molecular Research Center, Inc., Cincinnati, OH). 5 to 7.5 μg of total RNA was fractionated in a 1% agarose gel containing formaldehyde cast in a Wide Mini-Sub Cell gel tray (Bio-Rad, Hercules, CA) as described (Sambrook, et al.) with slight modifications. The formaldehyde concentration was reduced to 0.5M and the RNA was stained prior to electrophoresis with 100 μg/ml of etidium bromide that was added to the loading buffer. After electrophoresis with continuous buffer recirculation (60 volts/90 min), the gel was photographed and the RNA was transferred quantitatively to Zeta-probe nylon membrane (Biorad, Hercules, CA) by vacuum-blotting with 25

mM NaOH for 90 min. After neutralization for 5-10 min, with 1M Tris-HCl, pH 7.5 containing 3M NaCl, the blots were prehybridized with 50% formamide, 8% dextran sulfate, 6xSSPE, 0.1% SDS and 100 μg/ml of sheared and denatured salmon sperm DNA for at least 30 min at 42°C. cDNA inserts labeled with ^{32}P-dCTP by random priming (Stratagene, La Jolla, CA), were denatured with 0.25M NaOH (10 min at 37°C) and added to the prehybridization solution. After 24-65 hr at 42°C, the blots were washed under high stringency conditions (Sambrook, et al.) and exposed to X-ray films.

Results

Expression of DR3 was assessed by Northern blot in the following cell lines: TF274 (bone marrow stromal); MG63, TE85 (osteosarcoma); K562 (erythroid); KG1a, KG1, PLB985, HL60, U937, TNHP-1 (myeloid); REH, BJAB, Raji, IM-9 (B cell); Sup-T1, Jurkat, H9, Molt-3 (T cell); RL95-2 (endometrial carcinoma); MCF-7 (breast cancer); BE, HT29 (colon cancer); IMR32 (neuroblastoma) and could only be detected in KG1a cells. DR3 expression was detected in several lymphoblast cell lines. In the purified human hematopoietic cell populations, DR3 was weakly expressed in CD19+ cells, and more highly expressed in monocytes. However the highest levels were observed in T cells (CD4+ or CD8+) upon stimulation with PMA and PHA, indicating that DR3 probably plays a role in the regulation of T cell activation.

Example 5

Intracellular Signaling Molecules used by DR3 Protein

In vitro and *in vivo* binding studies were undertaken to investigate DR3 signaling pathways. Since DR3 contains a death domain, the inventors postulated that DR3, like TNFR-1 and Fas/APO-1, may transduce signals by recruiting death domain-containing adapter molecules (DAMs) such as FADD, TRADD, and RIP.

Experimental Design

In vitro binding experiments were performed as described previously (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)). Briefly, the cytoplasmic domains of DR3 (amino acid residues 215-393 (Figure 2)) and the death domain mutant ΔDR3 (amino acid residues 215-321 (Figure 2)) were amplified by PCR using appropriate templates and primers into pGSTag. pGSTag and pGSTag-TNFR-1 were described previously (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)). GST and GST fusion proteins were prepared from E.coli strain BL21(DE3)pLysS using standard published procedures and the recombinant proteins immobilized onto glutathione-agarose beads. ^{35}S-Labeled FADD, RIP and TRADD were prepared by in vitro transcription-translation using the TNT or T7 or SP6-coupled reticulocyte lysate system from Promega according to manufacturer's instructions, using pcDNA3 AU1-FADD (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)), pRK myc-TRADD (H. Hsu, *et al., Cell 81*, 495-504 (1995)), or pRK myc-RIP (H. Hsu, *et al., Immunity 4*, 387-396 (1996)) as template. Following translation, equal amounts of total ^{35}S-labeled reticulocyte lysate were diluted into 150 μl GST binding buffer (50 mM Tris, pH 7.6, 120 mM NaCl, 1% NP-40) and incubated for 2 hrs. at 4°C with the various GST fusion proteins complexed to beads, following the beads were pelleted by plus centrifugation, washed three times in GST buffer, boiled in SDS-sample buffer and resolved on a 12.5% SDS-PAGE. Bound proteins were visualized following autoradioraphy at -80°C. *In vitro* translated ^{35}S-labeled RIP, TRADD and FADD were incubated with glutathione beads containing GST alone or GST fusions of the cytoplasmic domain of Fas, TNFR-1,DR3 (215-393), or DDR3 (215-321). After the beads were washed, retained proteins were analyzed by SDS-PAGE and autoradiography. The gel was Coomassie stained to monitor equivalency of loading.

To demonstrate the association of DR3 and TRADD in vivo, constructs encoding Flag-TNFR-1 and Flag-ΔTNFR-1 were used. The Flag-TNFR-1 and Flag-ΔTNFR-1 constructs were described elsewhere (A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). The constructs encoding Flag-TNFR-1 and Flag-ΔTNFR-1 were described elsewhere (A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). To facilitate epitope tagging, DR3 and ΔDR3 (1-321) were cloned into the IBI Kodak FLAG plasmid (pCMV1FLAG) utilizing the signal peptide provided by the vector. 293 cells (2 x 10^6/100mm plate) were grown in DMEM media containing 10% heat-inactivated fetal bovine serum containing penicillin G, streptomycin, glutamine, and non-essential amino acids. Cells were transfected using calcium phosphate precipitation with the constructs encoding the indicated proteins in combination with pcDNA3-CrmA (M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)) to prevent cell death and thus maintain protein expression. Cells were lysed in 1 ml lysis buffer (50mM Hepes, 150mM NaCl, 1mM EDTA, 1% NP-40, and a protease inhibitor cocktail). Lysates were immunoprecipitated with a control monoclonal antibody or anti-Flag antibody for at least 4 hrs, at 4°C as previously described (A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). The beads were washed with lysis buffer 3X, but int he case of TRADD binding, the NaCl concentration was adjusted to 1M. The precipitates were fractioned on 12.5% SDS-PAGE and transferred to nitrocellulose. Subsequent Western blotting was performed as described elsewhere (H. Hsu *et al., Cell 84*, 299-308 (1996); Chinnaiyan, A.M. *et al., J Biol Chem 271*, 4961-4965 (1996)). After 24-32 hrs, extracts were prepared and immunoprecipitated with a control monoclonal antibody or anti-Flag monoclonal antibody (IBI Kodak). Western analysis indicated that myc-TRADD and death receptor expression levels were similar in all samples. Coprecipitating myc-TRADD was detected by immunoblotting using an anti-myc HRP conjugated antibody (Boehringer Mannheim).

Results

As an initial screen, *in vitro* translated radiolabeled DAMs were precipitated with various glutathione S-transferase (GST) fusion proteins immobilized on glutathione-Sepharose beads. As predicted from previous studies (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); H. Hsu, *et al., Cell 81*, 495-504 (1995)), FADD associated with the GST-Fas cytoplasmic domain while TRADD associated with the GST-TNFR-1 cytoplasmic domain. In addition, there was a direct, albeit weak, interaction between RIP and GST-TNFR-1. Interestingly, GST-DDCR associated specifically with TRADD, but not FADD or RIP. Furthermore, a truncated death domain mutant of DR3 (GST-DDR3) failed to interact with TRADD. To demonstrate the association of DR3 and TRADD *in vivo*, 293 cells were transiently transfected with plasmids that direct the synthesis of myc-epitope tagged TRADD (myc-TRADD) and Flag-epitope tagged DR3 (Flag-DR3), Flag-TNFR-1 or mutants. Consistent with the *in vitro* binding study, TRADD specifically coprecipitated with DR3 and TNFR-1, but not with the death domain mutants, DDR3 and DTNFR-1. Thus, it appears that DR3, like TNFR-1, may activate downstream signaling cascades by virtue of its ability to recruit the adapter molecule TRADD.

Overexpression of TRADD induces apoptosis and NF-kB activation-two of the most important activities signaled by TNFR-1 (H. Hsu, *et al., Cell 81*, 495-504 (1995)). Upon oligomerization of TNFR-1 by trimeric TNF, TRADD is recruited to the receptor signaling complex (H. Hsu, *et al., Cell 84*, 299-308 (1996)). TRADD can then recruit the following signal transducing molecules: 1) TRAF2, a TNFR-2- and CD40 - associated molecule (M. Rothe, *et al., Cell 78*, 681-92 (1994); M. Rothe, *et al., Science 269*, 1424-1427 (1995)), that mediates NF-kB activation, 2) RIP, originally identified as a Fas/APO-1-interacting protein by two-hybrid analysis (B.Z. Stanger, *et al., Cell 81*, 513-23 (1995)), that mediates NF-kB activation and apoptosis (H. Hsu, *et al., Immunity 4*, 387-396 (1996)), and 3) FADD, a Fas/APO-1- associated molecule, that mediates

apoptosis (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J. Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)). Thus, the inventors demonstrate that RIP, TRAF2 and FADD could be co-immunoprecipitated with DR3. In 293 cells expressing DR3 and RIP, only a weak association could be detected between the two molecules. However, in the presence of TRADD, RIP association with DR3 was significantly enhanced. Likewise, very little TRAF2 directly co-precipitated with DR3 in 293 cells. However, when DR3 and TRAF2 were expressed in the presence of TRADD and RIP (both of which can bind TRAF2), an enhanced binding of TRAF2 to DR3 could be detected. A similar association between FADD and DR3 was also observed. In the presence of TRADD, FADD efficiently coprecipitated with DR3.

Previous studies demonstrated that FADD could recruit the ICE/CED-3-like protease FLICE to the Fas/APO-1 death inducing signaling complex (M. Muzio, *et al., Cell 85*, 817-827 (1996); M.P. Boldin, *et al., Cell 85*, 803-815 (1996)). To demonstrate that FLICE can associate with TNFR-1 and DR3, coprecipitation experiments in 293 cells were carried out. Interestingly, FLICE was found complexed to TNFR-1 and DR3. Co-transfection of TRADD and/or FADD failed to enhance the FLICE-TNFR-1/DR3 interaction, suggesting that endogenous amounts of these adapter molecules were sufficient to maintain this association.

Example 6

DR3 Induced Apoptosis and NF-kB Activation

Overexpression of Fas/APO-1 and TNFR-1 in mammalian cells mimics receptor activation (M. Muzio, *et al.*, *Cell 85*, 817-827 (1996); M. P. Boldin, *et al., Cell 85*, 803-815 (1996)). Thus, this system was utilized to study the functional role of DDCR. Ectopic expression of DR3 in MCF7 breast carcinoma cells and 293 human embryonic kidney cells induced rapid apoptosis.

Experimental Design

Cell death assays were performed essentially as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995); A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). Briefly, MCF-7 human breast carcinoma clonal cell lines stably transfected with either vector alone, a CrmA expression construct (M. Tewari, *et al., J Biol Chem 270*, 3255-60 (1995)), or FADD-DN expression construct (A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)) were transiently transfected with pCMV-β-galatosidase in the presence of a ten-fold excess of pcDNA3 expression constructs encoding the indicated proteins using lipofectamine (GIBCO-BRL). 293 cells were likewise transfected using the $CaPO_4$ method. The ICE family inhibitor z-VAD-fmk (Enzyme Systems Products, Dublin, CA) was added to the cells at a concentration of 10μM, 5 hrs after transfection. 32 hours following transfection, cells were fixed and stained with X-Gal as previously described (A.M. Chinnaiyan, *et al., Cell 81*, 505-12 (1995); M.P. Boldin, *et al., J Biol Chem 270*, 7795-8 (1995); F.C. Kischkel, *et al., EMBO 14*, 5579-5588 (1995)). The data (mean +/- SD) shown are the percentage of round blue cells among the total number of blue cells counted. Data were obtained from at least three independent experiments.

NF-kB luciferase assays were performed as described elsewhere (H. Hsu, *et al., Immunity 4*, 387-396 (1996); M.D. Adams, *et al., Nature 377*, 3-174 (1995); G.S. Feng, *et al., J Biol Chem 271*, 12129-32 (1996); M. Rothe, *et al., Cell 78*, 681-92 (1994); M. Rothe, *et al., Science 269*, 1424-1427 (1995); (A.M. Chinnaiyan, *et al., J Biol Chem 271*, 4961-4965 (1996)). Briefly, 293 cells were co-transfected by calcium phosphate precipitation with pCMV-β-galactosidase, E-selectin-luciferase reporter gene (M. Rothe, *et al., Cell 78*, 681-92 (1994); M. Rothe, *et al., Science 269*, 1424-1427 (1995)), the indicated death receptors, and the indicated dominant negative inhibitors. In addition, DR3 or DDR3 was cotransfected with the pLantern expression construct (GIBCO-BRL) which encodes green fluorescent protein (photographic inset). Cells were visualized by

fluorescence microscopy using a FITC range barrier filter cube. Nuclei of transfected cells were visualized by DAPI staining and the image overlaid. (Cell death assays were performed essentially as previously described (Chinnaiyan, *et al., Cell 81*:505-12 (1995); Boldin, *et al., J. Biol. Chem. 270*:7795-8 (1995); Kischkel, *et al., EMBO 14*:5579-5588 (1995)); (Chinnaiyan, *et al., J. Biol. Chem. 271*:4961-4965 (1996)). The dominant negative inhibitors were used at a 4-fold higher quantity than the death receptors. Total DNA was kept constant.

To show that DR3 induces NF-kB activation which is inhibitable by RIP-DN (Stanger, *et al., Cell 81*:513-23 (1995)) and TRAF2-DN (Hsu, *et al. Cell 81*:495-504 (1995)); (Rothe, *et al., Cell 78*:681-92 (1994); Rothe, *et al. Science 269*:1424-1427 (1995)), 293 cells were co-transfected with the indicated molecules and an NF-kB luciferase reporter plasmid (Rothe, *et al., Cell 78*:681-92 (1994); Rothe, *et al. Science 269*:1424-1427 (1995)) and luciferase activities subsequently determined. NF-κB luciferase assays were performed as described elsewhere ((Hsu, *et al., Immunity 4*:387-396 (1996)); (Adams, *et al., Nature 377*:3-174 (1995); Feng, *et al., J. Biol. Chem. 271*:12129-32 (1996)); (Rothe, *et al., Cell 78*:681-92 (1994); Rothe, *et al. Science 269*:1424-1427 (1995)); Chinnaiyan, *et al., J. Biol. Chem. 271*:4961-4965 (1996)). Briefly, 293 cells were co-transfected by calcium phosphate precipitation with pCMB-β-galactosidase, E-selectin-luciferase reporter gene (Rothe, *et al., Cell 78*:681-92 (1994); Rothe, *et al. Science 269*:1424-1427 (1995)), the indicated death receptors, and the indicated dominant negative inhibitors. The dominant negative inhibitors were used at a 4-fold higher quantity than the death receptors. Total DNA was kept constant. Representative experiment performed in duplicate three independent times (mean ± SD).

Results

The cells displayed morphological alterations typical of cells undergoing apoptosis, becoming rounded, condensed and detaching from the dish. In MCF7 cells, plasmids encoding full-length DR3 or DDR3 were co-transfected with the pLantern reporter construct encoding green fluorescent protein. Nuclei of cells

transfected with DR3, but not DDR3, exhibited apoptotic morphology as assessed by DAPI staining. Similar to TNFR-1 and Fas/APO-1 (M. Muzio, *et al.*, *Cell 85*, 817-827 (1996); M. P. Boldin, *et al.*, *Cell 85*, 803-815 (1996); M. Tewari, *et al.*, *J Biol Chem 270*, 3255-60 (1995)), DR3-induced apoptosis was blocked by the inhibitors of ICE-like proteases, CrmA and z-VAD-fmk. Importantly, apoptosis induced by DR3 was also blocked by dominant negative versions of FADD (FADD-DN) or FLICE (FLICE-DN/MACHa1C360S), which were previously shown to inhibit death signaling by Fas/APO-1 and TNFR-1 (M. Muzio, *et al.*, *Cell 85*, 817-827 91996); M. P. Boldin, *et al.*, *Cell 85*, 803-815 (1996); H. Hsu, *et al.*, *Cell 84*, 299-398 (1996); A.M. Chinnaiyan, *et al.*, *J Biol Chem 271*, 4961-4965 (1996)). Thus, FADD and the ICE-like protease FLICE are likely necessary components of DR3-induced apoptosis.

As DR3 activation recruits three molecules implicated in TNF-induced NF-kB activation, we examined whether DR3 could activate NF-kB. Transfection of a control vector or expression of Fas/APO-1 failed to induce NF-kB activation. By contrast, NF-kB was activated by ectopic expression of DR3 or TNFR-1, but not by the inactive signaling mutants DDR3 or DTNFR-1. Importantly, DR3-induced NF-kB activation was blocked by dominant negative derivatives of RIP (RIP-DN) and TRAF2 (TRAF2-DN), which were previously shown to abrogate TNF-induced NF-kB activation (H. Hsu, *et al.*, *Cell 84*, 299-398 (1996); H. Hsu, *et al.*, *Immunity 4*, 387-396 (1996)). As expected, FADD-DN did not interfere with DR3-mediated NF-kB activation (H. Hsu, *et al.*, *Cell 84*, 299-398 (1996); A.M. Chinnaiyan, *et al.*, *J Biol Chem 271*, 4961-4965 (1996)).

Thus, the experiments set forth in Examples 6 and 7 demonstrate that DR3 is a death domain-containing molecule capable of triggering both apoptosis and NF-kB activation, two pathways dominant in the regulation of the immune system. The experiments also demonstrate the internal signal transduction machinery of this novel cell death receptor. The DR3 signaling complex assembles in a hierarchical manner with the recruitment of the multivalent

1) NF-kB activation mediated by TRAF2 and RIP and 2) cell death mediated by FADD, FLICE, and RIP.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosures of all patents, patent applications, and publications referred to herein are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1783 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 198..1481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CATGGGTGGG GGTGGGGGCG CTGCTGGATT CCTGCTCTGG TGGAGGGGAA ACTTGTGAGG      60

GGCTGGTAAG CGCCCCCTCC GAAGCCTGGT GTGTGCGCGG GGGGAAGGAA GTTAGTTTCC     120

TCTCCACCCA TGGGCACCCC TTCTGCCCGG GGCCTGGGAA GTGGGCTGCT CTGTGGGCAA     180

ATGCTGGGGC CTCTGAA ATG GAG GAG ACG CAG CAG GGA GAG GCC CCA CGT          230
                  Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg
                    1               5                  10

GGG CAG CTG CGC GGA GAG TCA GCA GCA CCT GTC CCC CAG GCG CTC CTC        278
Gly Gln Leu Arg Gly Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu
            15                  20                  25

CTG GTG CTG CTG GGG GCC CGG GCC CAG GGC GGC ACT CGT AGC CCC AGG        326
Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
        30                  35                  40

TGT GAC TGT GCC GGT GAC TTC CAC AAG AAG ATT GGT CTG TTT TGT TGC        374
Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
    45                  50                  55

AGA GGC TGC CCA GCG GGG CAC TAC CTG AAG GCC CCT TGC ACG GAG CCC        422
Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
60                  65                  70                  75

TGC GGC AAC TCC ACC TGC CTT GTG TGT CCC CAA GAC ACC TTC TTG GCC        470
Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
                80                  85                  90

TGG GAG AAC CAC CAT AAT TCT GAA TGT GCC CGC TGC CAG GCC TGT GAT        518
Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
            95                  100                 105

GAG CAG GCC TCC CAG GTG GCG CTG GAG AAC TGT TCA GCA GTG GCC GAC        566
Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
        110                 115                 120

ACC CGC TGT GGC TGT AAG CCA GGC TGG TTT GTG GAG TGC CAG GTC AGC        614
Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
    125                 130                 135

CAA TGT GTC AGC AGT TCA CCC TTC TAC TGC CAA CCA TGC CTA GAC TGC        662
Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
140                 145                 150                 155

GGG GCC CTG CAC CGC CAC ACA CGG CTA CTC TGT TCC CGC AGA GAT ACT        710
Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
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GAC TGT GGG ACC TGC CTG CCT GGC TTC TAT GAA CAT GGC GAT GGC TGC      758
Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
            175                 180                 185

GTG TCC TGC CCC ACG AGC ACC CTG GGG AGC TGT CCA GAG CGC TGT GCC      806
Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
        190                 195                 200

GCT GTC TGT GGC TGG AGG CAG ATG TTC TGG GTC CAG GTG CTC CTG GCT      854
Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
    205                 210                 215

GGC CTT GTG GTC CCC CTC CTG CTT GGG GCC ACC CTG ACC TAC ACA TAC      902
Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
220                 225                 230                 235

CGC CAC TGC TGG CCT CAC AAG CCC CTG GTT ACT GCA GAT GAA GCT GGG      950
Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
                240                 245                 250

ATG GAG GCT CTG ACC CCA CCA CCG GCC ACC CAT CTG TCA CCC TTG GAC      998
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            255                 260                 265

AGC GCC CAC ACC CTT CTA GCA CCT CCT GAC AGC AGT GAG AAG ATC TGC     1046
Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
        270                 275                 280

ACC GTC CAG TTG GTG GGT AAC AGC TGG ACC CCT GGC TAC CCC GAG ACC     1094
Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
    285                 290                 295

CAG GAG GCG CTC TGC CCG CAG GTG ACA TGG TCC TGG GAC CAG TTG CCC     1142
Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
300                 305                 310                 315

AGC AGA GCT CTT GGC CCC GCT GCT GCG CCC ACA CTC TCG CCA GAG TCC     1190
Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
                320                 325                 330

CCA GCC GGC TCG CCA GCC ATG ATG CTG CAG CCG GGC CCG CAG CTC TAC     1238
Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
            335                 340                 345

GAC GTG ATG GAC GCG GTC CCA GCG CGG CGC TGG AAG GAG TTC GTG CGC     1286
Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
        350                 355                 360

ACG CTG GGG CTG CGC GAG GCA GAG ATC GAA GCC GTG GAG GTG GAG ATC     1334
Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
    365                 370                 375

GGC CGC TTC CGA GAC CAG CAG TAC GAG ATG CTC AAG CGC TGG CGC CAG     1382
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Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
380                 385                 390                 395

CAG CAG CCC GCG GGC CTC GGA GCC GTT TAC GCG GCC CTG GAG CGC ATG      1430
Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
                400                 405                 410

GGG CTG GAC GGC TGC GTG GAA GAC TTG CGC AGC CGC CTG CAG CGC GGC      1478
Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
            415                 420                 425

CCG TGACACGGCG CCCACTTGCC ACCTAGGCGC TCTGGTGGCC CTTGCAGAAG           1531
Pro

CCCTAAGTAC GGTTACTTAT GCGTGTAGAC ATTTTATGTC ACTTATTAAG CCGCTGGCAC    1591

GGCCCTGCGT AGCAGCACCA GCCGGCCCCA CCCCTGCTCG CCCCTATCGC TCCAGCCAAG    1651

GCGAAGAAGC ACGAACGAAT GTCGAGAGGG GGTGAAGACA TTTCTCAACT TCTCGGCCGG    1711

AGTTTGGCTG AGATCGCGGT ATTAAATCTG TGAAAGAAAA CAAAACAAAA CAAAAAAAAA    1771

AAAAAAAAAA AA                                                        1783
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg Gly Gln Leu Arg Gly
 1               5                  10                  15

Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu Leu Val Leu Leu Gly
            20                  25                  30

Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg Cys Asp Cys Ala Gly
        35                  40                  45

Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys Arg Gly Cys Pro Ala
    50                  55                  60

Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro Cys Gly Asn Ser Thr
65                  70                  75                  80

Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala Trp Glu Asn His His
                85                  90                  95
```

```
Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp Glu Gln Ala Ser Gln
            100                 105                 110

Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp Thr Arg Cys Gly Cys
        115                 120                 125

Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser Gln Cys Val Ser Ser
    130                 135                 140

Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys Gly Ala Leu His Arg
145                 150                 155                 160

His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr Asp Cys Gly Thr Cys
                165                 170                 175

Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys Val Ser Cys Pro Thr
            180                 185                 190

Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala Ala Val Cys Gly Trp
        195                 200                 205

Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala Gly Leu Val Val Pro
    210                 215                 220

Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr Arg His Cys Trp Pro
225                 230                 235                 240

His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly Met Glu Ala Leu Thr
                245                 250                 255

Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp Ser Ala His Thr Leu
            260                 265                 270

Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys Thr Val Gln Leu Val
        275                 280                 285

Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr Gln Glu Ala Leu Cys
    290                 295                 300

Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro Ser Arg Ala Leu Gly
305                 310                 315                 320

Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser Pro Ala Gly Ser Pro
                325                 330                 335

Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr Asp Val Met Asp Ala
            340                 345                 350

Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg Thr Leu Gly Leu Arg
        355                 360                 365

Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile Gly Arg Phe Arg Asp
    370                 375                 380

Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln Gln Gln Pro Ala Gly
```

385 390 395 400

Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met Gly Leu Asp Gly Cys
405 410 415

Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly Pro
420 425

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1254 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1251

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG GAG CAG CGG CCG CGG GGC TGC GCG GCG GTG GCG GCG GCG CTC CTC      48
Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu Leu
 430                 435                 440

CTG GTG CTG CTG GGG GCC CGG GCC CAG GGC GGC ACT CGT AGC CCC AGG      96
Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
445                 450                 455                 460

TGT GAC TGT GCC GGT GAC TTC CAC AAG AAG ATT GGT CTG TTT TGT TGC     144
Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
                465                 470                 475

AGA GGC TGC CCA GCG GGG CAC TAC CTG AAG GCC CCT TGC ACG GAG CCC     192
Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
            480                 485                 490

TGC GGC AAC TCC ACC TGC CTT GTG TGT CCC CAA GAC ACC TTC TTG GCC     240
Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
        495                 500                 505

TGG GAG AAC CAC CAT AAT TCT GAA TGT GCC CGC TGC CAG GCC TGT GAT     288
Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
 510                 515                 520

GAG CAG GCC TCC CAG GTG GCG CTG GAG AAC TGT TCA GCA GTG GCC GAC     336
Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
525                 530                 535                 540

ACC CGC TGT GGC TGT AAG CCA GGC TGG TTT GTG GAG TGC CAG GTC AGC     384
```

```
Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
                545                 550                 555

CAA TGT GTC AGC AGT TCA CCC TTC TAC TGC CAA CCA TGC CTA GAC TGC      432
Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
            560                 565                 570

GGG GCC CTG CAC CGC CAC ACA CGG CTA CTC TGT TCC CGC AGA GAT ACT      480
Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
        575                 580                 585

GAC TGT GGG ACC TGC CTG CCT GGC TTC TAT GAA CAT GGC GAT GGC TGC      528
Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
    590                 595                 600

GTG TCC TGC CCC ACG AGC ACC CTG GGG AGC TGT CCA GAG CGC TGT GCC      576
Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
605                 610                 615                 620

GCT GTC TGT GGC TGG AGG CAG ATG TTC TGG GTC CAG GTG CTC CTG GCT      624
Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
                625                 630                 635

GGC CTT GTG GTC CCC CTC CTG CTT GGG GCC ACC CTG ACC TAC ACA TAC      672
Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
            640                 645                 650

CGC CAC TGC TGG CCT CAC AAG CCC CTG GTT ACT GCA GAT GAA GCT GGG      720
Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
        655                 660                 665

ATG GAG GCT CTG ACC CCA CCA CCG GCC ACC CAT CTG TCA CCC TTG GAC      768
Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
    670                 675                 680

AGC GCC CAC ACC CTT CTA GCA CCT CCT GAC AGC AGT GAG AAG ATC TGC      816
Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
685                 690                 695                 700

ACC GTC CAG TTG GTG GGT AAC AGC TGG ACC CCT GGC TAC CCC GAG ACC      864
Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
                705                 710                 715

CAG GAG GCG CTC TGC CCG CAG GTG ACA TGG TCC TGG GAC CAG TTG CCC      912
Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
            720                 725                 730

AGC AGA GCT CTT GGC CCC GCT GCT GCG CCC ACA CTC TCG CCA GAG TCC      960
Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
        735                 740                 745

CCA GCC GGC TCG CCA GCC ATG ATG CTG CAG CCG GGC CCG CAG CTC TAC     1008
Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
    750                 755                 760
```

```
GAC GTG ATG GAC GCG GTC CCA GCG CGG CGC TGG AAG GAG TTC GTG CGC      1056
Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
765                 770                 775                 780

ACG CTG GGG CTG CGC GAG GCA GAG ATC GAA GCC GTG GAG GTG GAG ATC      1104
Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
                785                 790                 795

GGC CGC TTC CGA GAC CAG CAG TAC GAG ATG CTC AAG CGC TGG CGC CAG      1152
Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
            800                 805                 810

CAG CAG CCC GCG GGC CTC GGA GCC GTT TAC GCG GCC CTG GAG CGC ATG      1200
Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
        815                 820                 825

GGG CTG GAC GGC TGC GTG GAA GAC TTG CGC AGC CGC CTG CAG CGC GGC      1248
Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
    830                 835                 840

CCG TGA                                                             1254
Pro
845
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 417 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Glu Gln Arg Pro Arg Gly Cys Ala Ala Val Ala Ala Ala Leu Leu
  1               5                  10                  15

Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
             20                  25                  30

Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
         35                  40                  45

Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
     50                  55                  60

Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
 65                  70                  75                  80

Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
                 85                  90                  95

Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
```

```
                100                 105                 110

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
        115                 120                 125

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
    130                 135                 140

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
145                 150                 155                 160

Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
                165                 170                 175

Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
            180                 185                 190

Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
        195                 200                 205

Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
    210                 215                 220

Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
225                 230                 235                 240

Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
                245                 250                 255

Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
            260                 265                 270

Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
        275                 280                 285

Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
    290                 295                 300

Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
305                 310                 315                 320

Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
                325                 330                 335

Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
            340                 345                 350

Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
        355                 360                 365

Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
    370                 375                 380

Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
385                 390                 395                 400
```

Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
405 410 415

Pro

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 455 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr

```
                180                 185                 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
        195                 200                 205

Gly Thr Thr Val Leu Leu Pro Leu Val Ile Phe Phe Gly Leu Cys Leu
    210                 215                 220

Leu Ser Leu Leu Phe Ile Gly Leu Met Tyr Arg Tyr Gln Arg Trp Lys
225                 230                 235                 240

Ser Lys Leu Tyr Ser Ile Val Cys Gly Lys Ser Thr Pro Glu Lys Glu
                245                 250                 255

Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
            260                 265                 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
        275                 280                 285

Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys
    290                 295                 300

Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
305                 310                 315                 320

Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
                325                 330                 335

Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
            340                 345                 350

Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
        355                 360                 365

Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
    370                 375                 380

Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
385                 390                 395                 400

Tyr Ser Met Leu Ala Thr Trp Arg Arg Arg Thr Pro Arg Arg Glu Ala
                405                 410                 415

Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
            420                 425                 430

Cys Leu Glu Asp Ile Glu Glu Ala Leu Cys Gly Pro Ala Ala Leu Pro
        435                 440                 445

Pro Ala Pro Ser Leu Leu Arg
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 335 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1               5                  10                  15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
            20                  25                  30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
        35                  40                  45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
    50                  55                  60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65                  70                  75                  80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
                85                  90                  95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
            100                 105                 110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
        115                 120                 125

Cys Lys Pro Asn Phe Phe Gln Asn Ser Thr Val Cys Glu His Cys Asp
    130                 135                 140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145                 150                 155                 160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
                165                 170                 175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
            180                 185                 190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
        195                 200                 205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
    210                 215                 220
```

Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225 230 235 240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
245 250 255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
260 265 270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
275 280 285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
290 295 300

Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
305 310 315 320

Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
325 330 335

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCGCCATGGG GGCCCGGCGG CAG 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGAAGCTTC TAGGACCCAG AACATCTGCC 30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGCGGATCCG CCATCATGGA GGAGACGCAG CAG 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCG CCATCATGGA GCAGCGGCCG CGG 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCGTCTAGAT CAAAGCGTAG TCTGGGACGT CGTATGGGTA CGGGCCGCGC TGCA 54

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCGGATCCG CCATCATGGA GGAGACGCAG CAG 33

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCGGATCCG CCATCATGGA GCAGCGGCCG CGG 33

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGCGGATCCT CACGGGCCGC GCTGCA 26

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGATCCG CCATCATGGA GGAGACGCAG CAG 33

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGGATCCG CCATCATGGA GCAGCGGCCG CGG 33

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCGAGATCTA GTCTGGACCC AGAACATCTG CCTCC 35

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding the full-length death domain containing receptor polypeptide (DR3-V1) having the complete amino acid sequence in Figure 1 (SEQ ID NO:2);

(b) nucleotide sequence encoding the full-length death domain containing receptor (DR3) polypeptide having the complete amino acid sequence in Figure 2 (SEQ ID NO:4), including the predicted leader sequence;

(c) a nucleotide sequence encoding the DR3-V1 polypeptide having the amino acid sequence at positions from about 36 to about 428 in Figure 1 (SEQ ID NO:2);

(d) a nucleotide sequence encoding the full-length DR3-V1 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No. 97456;

(e) a nucleotide sequence encoding the full-length DR3 polypeptide having the complete amino acid sequence including the leader encoded by the cDNA clone contained in ATCC Deposit No._____;

(f) a nucleotide sequence encoding the mature DR3-V1 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97456;

(g) a nucleotide sequence encoding the mature DR3 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No._____;

(h) a nucleotide sequence that encodes the DR3 extracellular domain;

(i) a nucleotide sequence that encodes the DR3 transmembrane domain;

(j) a nucleotide sequence that encodes the DR3 intracellular domain;

(k) a nucleotide sequence that encodes the DR3 death domain; and

(l) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k).

2. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 1 (SEQ ID NO:1).

3. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the death domain containing receptor polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2).

4. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the mature death domain containing receptor polypeptide having the amino acid sequence in Figure 1 (SEQ ID NO:2).

5. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. 97456.

6. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence in Figure 2 (SEQ ID NO:3).

7. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 1 (SEQ ID NO:1) encoding the death domain containing receptor polypeptide having the complete amino acid sequence in Figure 1 (SEQ ID NO:2).

8. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence in Figure 2 (SEQ ID NO:3) encoding the mature death domain containing receptor polypeptide having the amino acid sequence in Figure 2 (SEQ ID NO:4).

9. The nucleic acid molecule of claim 1 wherein said polynucleotide has the complete nucleotide sequence of the cDNA clone contained in ATCC Deposit No. _____.

10. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the death domain containing receptor polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97456.

11. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature death domain containing receptor polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97456.

12. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the death domain containing receptor polypeptide having the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. _____.

13. The nucleic acid molecule of claim 1 wherein said polynucleotide has the nucleotide sequence encoding the mature death domain containing receptor polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No._____.

14. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k) or (l) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

15. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a death domain containing receptor polypeptide having an amino acid sequence in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k) of claim 1.

16. The isolated nucleic acid molecule of claim 15, which encodes an epitope-bearing portion of a death domain containing receptor polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about 1 to about 22 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 33 to about 56 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 59 to about 82 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 95 to about 112 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 122 to about 133 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 161 to about 177 in Figure 1 (SEQ ID NO:2); a polypeptide comprising amino acid residues from about 179 to about 190 in Figure 1 (SEQ ID NO:2); and a polypeptide comprising amino acid residues from about 196 to about 205 in Figure 1 (SEQ ID NO:2).

17. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

18. A recombinant vector produced by the method of claim 17.

19. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 18 into a host cell.

20. A recombinant host cell produced by the method of claim 19.

21. A recombinant method for producing a death domain containing receptor polypeptide, comprising culturing the recombinant host cell of claim 20 under conditions such that said polypeptide is expressed and recovering said polypeptide.

22. An isolated death domain containing receptor polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) the amino acid sequence of the DR3-V1 polypeptide having the complete 428 amino acid sequence, including the leader sequence shown in Figure 1 (SEQ ID NO:2);

(b) the amino acid sequence of the DR3 polypeptide having the complete 417 amino acid sequence, including the leader sequence, shown in Figure 2 (SEQ ID NO:4);

(c) the amino acid sequence of the DR3-V1 polypeptide having the amino acid sequence at positions from about 36 to about 428 in Figure 1 (SEQ ID NO:2);

(d) the amino acid sequence of the DR3-V1 polypeptide having the complete amino acid sequence, including the leader, encoded by the cDNA clone contained in ATCC Deposit No. 97456;

(e) the amino acid sequence of the DR3 polypeptide having the complete amino acid sequence, including the leader, encoded by the cDNA clone contained in ATCC Deposit No. _____;

(f) the amino acid sequence of the mature DR3 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97456;

(g) the amino acid sequence encoding the mature DR3 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. _____;

(h) the amino acid sequence of the DR3 extracellular domain;

(i) the amino acid sequence of the DR3 transmembrane domain;

(j) the amino acid sequence of the DR3 intracellular domain;

(k) the amino acid sequence of the DR3 death domain; and

(l) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k).

23. An isolated antibody that binds specifically to a death domain containing receptor polypeptide of claim 22.

24. A method of treating diseases and disorders associated with the inhibition of apoptosis comprising administering an effective amount of the polypeptide as claimed in claim 22, or an agonist thereof to a patient in need thereof.

25. A method of treating diseases and disorders associated with increased apoptosis comprising administering to a patient in need thereof an effective amount of an antagonist of the polypeptide as claimed in claim 22 to a patient in need thereof.

26. A method of treating inflammatory diseases and disorders comprising administering to a patient in need thereof an effective amount of an antagonist of the polypeptide as claimed in claim 22.

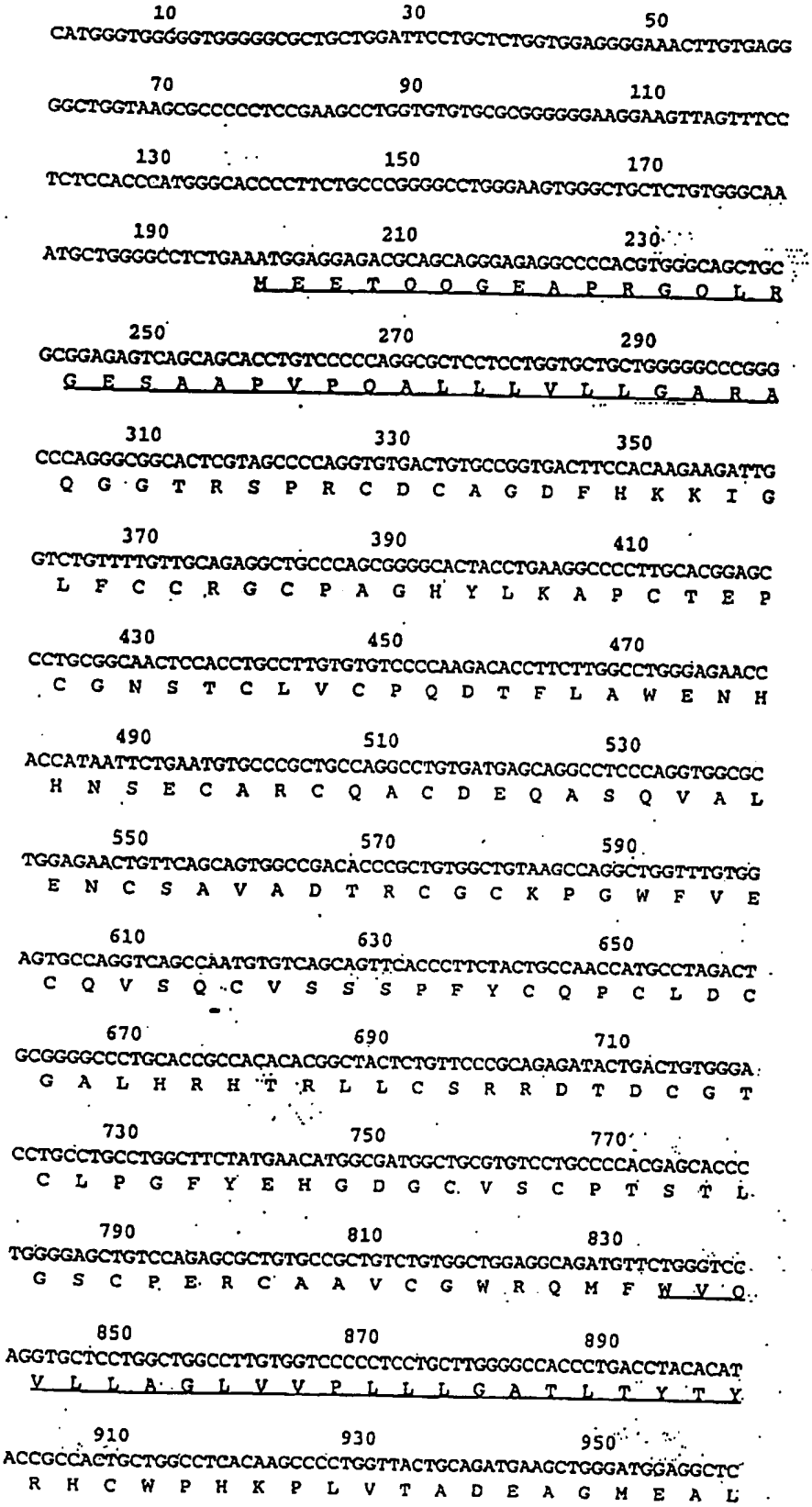
10 30 50
CATGGGTGGGGGTGGGGGCGCTGCTGGATTCCTGCTCTGGTGGAGGGGAAACTTGTGAGG
70 90 110
GGCTGGTAAGCGCCCCCTCCGAAGCCTGGTGTGTGCGCGGGGGGAAGGAAGTTAGTTTCC
130 150 170
TCTCCACCCATGGGCACCCCTTCTGCCCGGGGCCTGGGAAGTGGGCTGCTCTGTGGGCAA
190 210 230
ATGCTGGGGCCTCTGAAATGGAGGAGACGCAGCAGGGAGAGGCCCCACGTGGGCAGCTGC
M E E T Q Q G E A P R G Q L R
250 270 290
GCGGAGAGTCAGCAGCACCTGTCCCCCAGGCGCTCCTCCTGGTGCTGCTGGGGGCCCGGG
G E S A A P V P Q A L L L V L L G A R A
310 330 350
CCCAGGGCGGCACTCGTAGCCCCAGGTGTGACTGTGCCGGTGACTTCCACAAGAAGATTG
Q G G T R S P R C D C A G D F H K K I G
370 390 410
GTCTGTTTTGTTGCAGAGGCTGCCCAGCGGGGCACTACCTGAAGGCCCCTTGCACGGAGC
L F C C R G C P A G H Y L K A P C T E P
430 450 470
CCTGCGGCAACTCCACCTGCCTTGTGTGTCCCCAAGACACCTTCTTGGCCTGGGAGAACC
C G N S T C L V C P Q D T F L A W E N H
490 510 530
ACCATAATTCTGAATGTGCCCGCTGCCAGGCCTGTGATGAGCAGGCCTCCCAGGTGGCGC
H N S E C A R C Q A C D E Q A S Q V A L
550 570 590
TGGAGAACTGTTCAGCAGTGGCCGACACCCGCTGTGGCTGTAAGCCAGGCTGGTTTGTGG
E N C S A V A D T R C G C K P G W F V E
610 630 650
AGTGCCAGGTCAGCCAATGTGTCAGCAGTTCACCCTTCTACTGCCAACCATGCCTAGACT
C Q V S Q C V S S S P F Y C Q P C L D C
670 690 710
GCGGGGCCCTGCACCGCCACACACGGCTACTCTGTTCCCGCAGAGATACTGACTGTGGGA
G A L H R H T R L L C S R R D T D C G T
730 750 770
CCTGCCTGCCTGGCTTCTATGAACATGGCGATGGCTGCGTGTCCTGCCCCACGAGCACCC
C L P G F Y E H G D G C V S C P T S T L
790 810 830
TGGGGAGCTGTCCAGAGCGCTGTGCCGCTGTCTGTGGCTGGAGGCAGATGTTCTGGGTCC
G S C P E R C A A V C G W R Q M F W V Q
850 870 890
AGGTGCTCCTGGCTGGCCTTGTGGTCCCCCTCCTGCTTGGGGCCACCCTGACCTACACAT
V L L A G L V V P L L L G A T L T Y T Y
910 930 950
ACCGCCACTGCTGGCCTCACAAGCCCCTGGTTACTGCAGATGAAGCTGGGATGGAGGCTC
R H C W P H K P L V T A D E A G M E A L

FIGURE 1A

```
      970                  990                 1010
TGACCCCACCACCGGCCACCCATCTGTCACCCTTGGACAGCGCCCACACCCTTCTAGCAC
  T  P  P  P  A  T  H  L  S  P  L  D  S  A  H  T  L  L  A  P

     1030                 1050                1070
CTCCTGACAGCAGTGAGAAGATCTGCACCGTCCAGTTGGTGGGTAACAGCTGGACCCCTG
  P  D  S  S  E  K  I  C  T  V  Q  L  V  G  N  S  W  T  P  G

     1090                 1110                1130
GCTACCCCGAGACCCAGGAGGCGCTCTGCCCGCAGGTGACATGGTCCTGGGACCAGTTGC
  Y  P  E  T  Q  E  A  L  C  P  Q  V  T  W  S  W  D  Q  L  P

     1150                 1170                1190
CCAGCAGAGCTCTTGGCCCCGCTGCTGCGCCCACACTCTCGCCAGAGTCCCCAGCCGGCT
  S  R  A  L  G  P  A  A  A  P  T  L  S  P  E  S  P  A  G  S

     1210                 1230                1250
CGCCAGCCATGATGCTGCAGCCGGGCCCGCAGCTCTACGACGTGATGGACGCGGTCCCAG
  P  A  M  M  L  Q  P  G  P  Q  L  Y  D  V  M  D  A  V  P  A

     1270                 1290                1310
CGCGGCGCTGGAAGGAGTTCGTGCGCACGCTGGGGCTGCGCGAGGCAGAGATCGAAGCCG
  R  R  W  K  E  F  V  R  T  L  G  L  R  E  A  E  I  E  A  V

     1330                 1350                1370
TGGAGGTGGAGATCGGCCGCTTCCGAGACCAGCAGTACGAGATGCTCAAGCGCTGGCGCC
  E  V  E  I  G  R  F  R  D  Q  Q  Y  E  M  L  K  R  W  R  Q

     1390                 1410                1430
AGCAGCAGCCCGCGGGCCTCGGAGCCGTTTACGCGGCCCTGGAGCGCATGGGGCTGGACG
  Q  Q  P  A  G  L  G  A  V  Y  A  A  L  E  R  M  G  L  D  G

     1450                 1470                1490
GCTGCGTGGAAGACTTGCGCAGCCGCCTGCAGCGCGGCCCGTGACACGGCGCCCACTTGC
  C  V  E  D  L  R  S  R  L  Q  R  G  P  *

     1510                 1530                1550
CACCTAGGCGCTCTGGTGGCCCTTGCAGAAGCCCTAAGTACGGTTACTTATGCGTGTAGA

     1570                 1590                1610
CATTTTATGTCACTTATTAAGCCGCTGGCACGGCCCTGCGTAGCAGCACCAGCCGGCCCC

     1630                 1650                1670
ACCCCTGCTCGCCCCTATCGCTCCAGCCAAGGCGAAGAAGCACGAACGAATGTCGAGAGG

     1690                 1710                1730
GGGTGAAGACATTTCTCAACTTCTCGGCCGGAGTTTGGCTGAGATCGCGGTATTAAATCT

     1750                 1770
GTGAAAGAAAACAAAACAAAACAAAAAAAAAAAAAAAAAAAA
```

FIGURE 1B

```
1     ATGGAGCAGC GGCCGCGGGG CTGCGCGGCG GTGGCGGCGG CGCTCCTCCT GGTGCTGCTG
      M  E  Q  R  P  R  G  C  A  A  V  A  A  A  L  L  L  V  L  L
61    GGGGCCCGGG CCCAGGGCGG CACTCGTAGC CCCAGGTGTG ACTGTGCCGG TGACTTCCAC
      G  A  R  A  Q  G  G  T  R  S  P  R  C  D  C  A  G  D  F  H
121   AAGAAGATTG GTCTGTTTTG TTGCAGAGGC TGCCCAGCGG GGCACTACCT GAAGGCCCCT
      K  K  I  G  L  F  C  C  R  G  C  P  A  G  H  Y  L  K  A  P
181   TGCACGGAGC CCTGCGGCAA CTCCACCTGC CTTGTGTGTC CCCAAGACAC CTTCTTGGCC
      C  T  E  P  C  G  N  S  T  C  L  V  C  P  Q  D  T  F  L  A
241   TGGGAGAACC ACCATAATTC TGAATGTGCC CGCTGCCAGG CCTGTGATGA GCAGGCCTCC
      W  E  N  H  H  N  S  E  C  A  R  C  Q  A  C  D  E  Q  A  S
301   CAGGTGGCGC TGGAGAACTG TTCAGCAGTG GCCGACACCC GCTGTGGCTG TAAGCCAGGC
      Q  V  A  L  E  N  C  S  A  V  A  D  T  R  C  G  C  K  P  G
361   TGGTTTGTGG AGTGCCAGGT CAGCCAATGT GTCAGCAGTT CACCCTTCTA CTGCCAACCA
      W  F  V  E  C  Q  V  S  Q  C  V  S  S  S  P  F  Y  C  Q  P
421   TGCCTAGACT GCGGGGCCCT GCACCGCCAC ACACGGCTAC TCTGTTCCCG CAGAGATACT
      C  L  D  C  G  A  L  H  R  H  T  R  L  L  C  S  R  R  D  T
481   GACTGTGGGA CCTGCCTGCC TGGCTTCTAT GAACATGGCG ATGGCTGCGT GTCCTGCCCC
      D  C  G  T  C  L  P  G  F  Y  E  H  G  D  G  C  V  S  C  P
541   ACGAGCACCC TGGGGAGCTG TCCAGAGCGC TGTGCCGCTG TCTGTGGCTG GAGGCAGATG
      T  S  T  L  G  S  C  P  E  R  C  A  A  V  C  G  W  R  Q  M
601   TTCTGGGTCC AGGTGCTCCT GGCTGGCCTT GTGGTCCCCC TCCTGCTTGG GGCCACCCTG
      F  W  V  Q  V  L  L  A  G  L  V  V  P  L  L  L  G  A  T  L
661   ACCTACACAT ACCGCCACTG CTGGCCTCAC AAGCCCCTGG TTACTGCAGA TGAAGCTGGG
      T  Y  T  Y  R  H  C  W  P  H  K  P  L  V  T  A  D  E  A  G
721   ATGGAGGCTC TGACCCCACC ACCGGCCACC CATCTGTCAC CCTTGGACAG CGCCCACACC
      M  E  A  L  T  P  P  P  A  T  H  L  S  P  L  D  S  A  H  T
781   CTTCTAGCAC CTCCTGACAG CAGTGAGAAG ATCTGCACCG TCCAGTTGGT GGGTAACAGC
      L  L  A  P  P  D  S  S  E  K  I  C  T  V  Q  L  V  G  N  S
841   TGGACCCCTG GCTACCCCGA GACCCAGGAG GCGCTCTGCC CGCAGGTGAC ATGGTCCTGG
      W  T  P  G  Y  P  E  T  Q  E  A  L  C  P  Q  V  T  W  S  W
901   GACCAGTTGC CCAGCAGAGC TCTTGGCCCC GCTGCTGCGC CCACACTCTC GCCAGAGTCC
      D  Q  L  P  S  R  A  L  G  P  A  A  A  P  T  L  S  P  E  S
961   CCAGCCGGCT CGCCAGCCAT GATGCTGCAG CCGGGCCCGC AGCTCTACGA CGTGATGGAC
      P  A  G  S  P  A  M  M  L  Q  P  G  P  Q  L  Y  D  V  M  D
1021  GCGGTCCCAG CGCGGCGCTG GAAGGAGTTC GTGCGCACGC TGGGGCTGCG CGAGGCAGAG
      A  V  P  A  R  R  W  K  E  F  V  R  T  L  G  L  R  E  A  E
1081  ATCGAAGCCG TGGAGGTGGA GATCGGCCGC TTCCGAGACC AGCAGTACGA GATGCTCAAG
      I  E  A  V  E  V  E  I  G  R  F  R  D  Q  Q  Y  E  M  L  K
1141  CGCTGGCGCC AGCAGCAGCC CGCGGGCCTC GGAGCCGTTT ACGCGGCCCT GGAGCGCATG
      R  W  R  Q  Q  Q  P  A  G  L  G  A  V  Y  A  A  L  E  R  M
1201  GGGCTGGACG GCTGCGTGGA AGACTTGCGC AGCCGCCTGC AGGGCGGCCC GTGA
      G  L  D  G  C  V  E  D  L  R  S  R  L  Q  R  G  P  .
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FIGURE 2

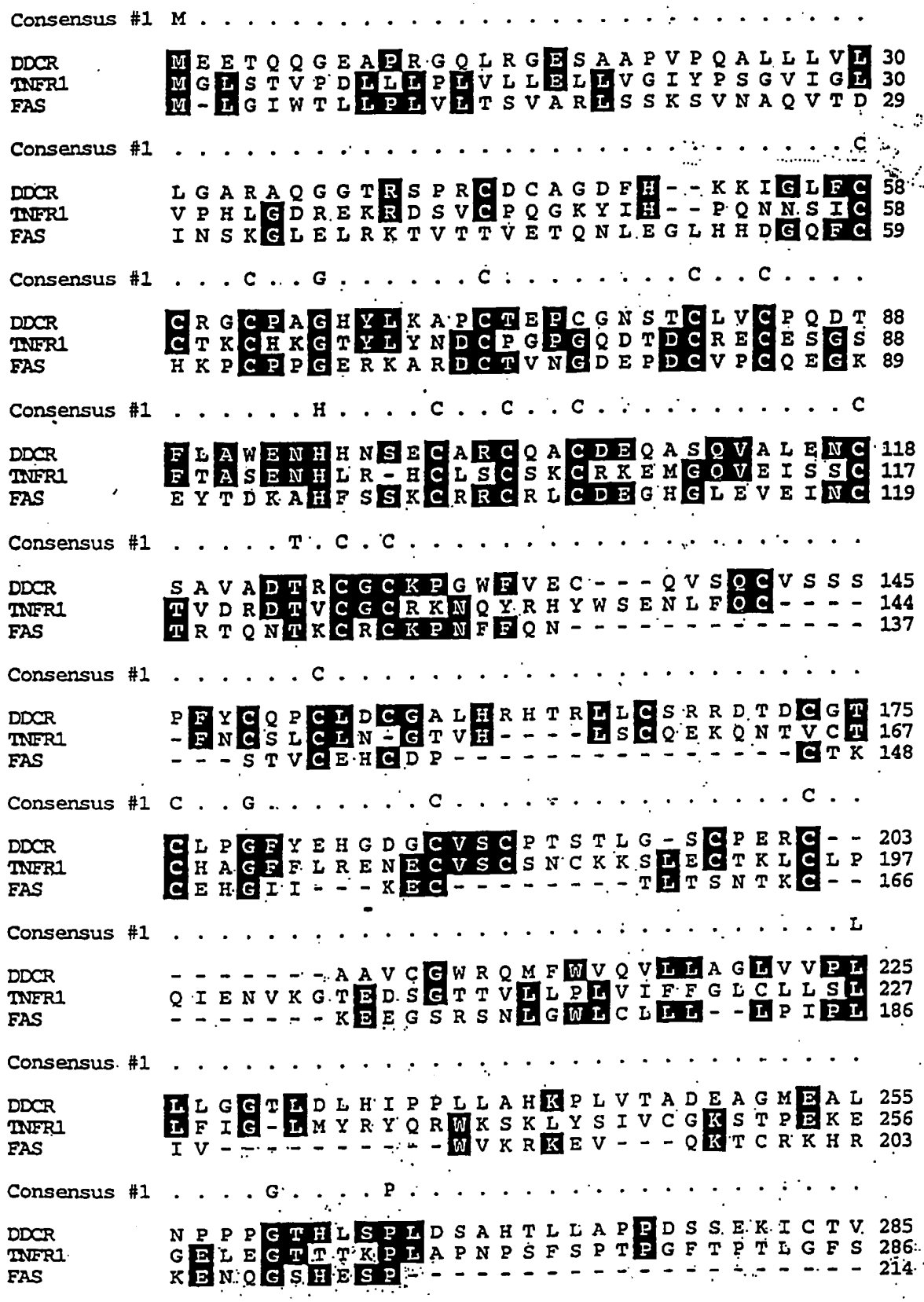
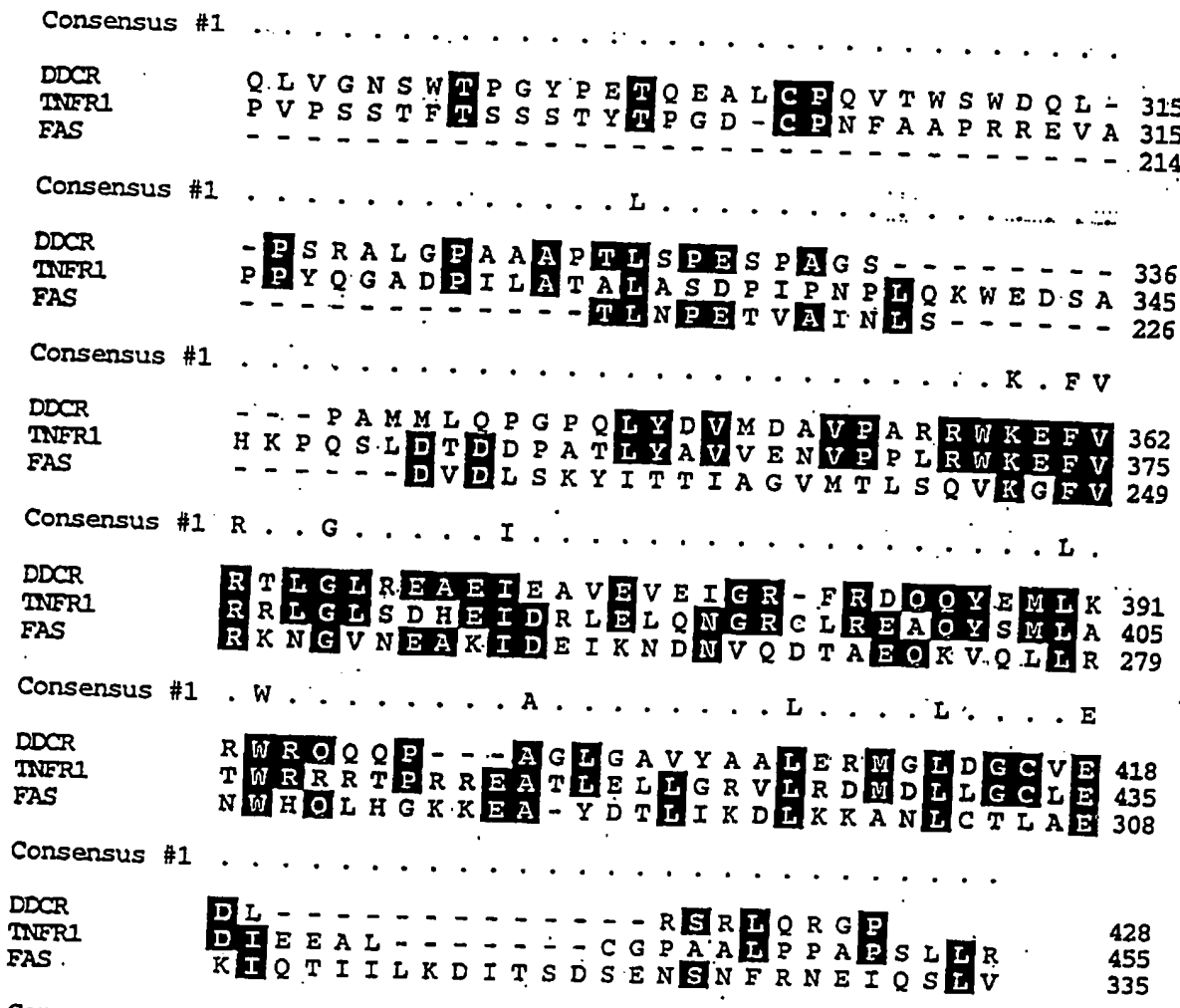

Consensus #1 M . . . . . .
DDCR MEETQQGEAPRGQLRGESAAPVPQALLLVL 30
TNFR1 MGLSTVPDLLLPLVLLELLVGIYPSGVIGL 30
FAS M-LGIWTLLPLVLTSVARLSSKSVNAQVTD 29
Consensus #1 C
DDCR LGARAQGGTRSPRCDCAGDFH--KKIGLFC 58
TNFR1 VPHLGDREKRDSVCPQGKYIH--PQNNSIC 58
FAS INSKGLELRKTVTTVETQNLEGLHHDGQFC 59
Consensus #1 . . . C . . G . . . . . C . . . . . C . . C . . . .
DDCR CRGCPAGHYLKAPCTEPCGNSTCLVCPQDT 88
TNFR1 CTKCHKGTYLYNDCPGPGQDTDCRECESGS 88
FAS HKPCPPGERKARDCTVNGDEPDCVPCQEGK 89
Consensus #1 . . . . . H . . . . C . . C . . C C
DDCR FLAWENHHNSECARCQACDEQASQVALENC 118
TNFR1 FTASENHLR-HCLSCSKCRKEMGQVEISSC 117
FAS EYTDKAHFSSKCRRCRLCDEGHGLEVEINC 119
Consensus #1 . . . . . T . C . C . . . . . .
DDCR SAVADTRCGCKPGWFVEC---QVSQCVSSS 145
TNFR1 TVDRDTVCGCRKNQYRHYWSENLFQC---- 144
FAS TRTQNTKCRCKPNFFQN---------------- 137
Consensus #1 . . . . . C . . . . . .
DDCR PFYCQPCLDCGALHRHTRLLCSRRDTDCGT 175
TNFR1 -FNCSLCLN-GTVH----LSCQEKQNTVCT 167
FAS ---STVCEHCDP----------CTK 148
Consensus #1 C . . G . . . . . C . . . . . C . .
DDCR CLPGFYEHGDGCVSCPTSTLG-SCPERC-- 203
TNFR1 CHAGFFLRENECVSCSNCKKSLECTKLCLP 197
FAS CEHGIIK-ECTLTSNTKC-- 166
Consensus #1 L
DDCR -------AAVCGWRQMFWVQVLLAGLVVPL 225
TNFR1 QIENVKGTEDSGTTVLLPLVIFFGLCLLSL 227
FAS -------KEEGSRSNLGWLCLLL--LPIPL 186
Consensus #1 . . . . . .
DDCR LLGGTLDLHIPPLLAHKPLVTADEAGMEAL 255
TNFR1 LFIG-LMYRYQRWKSKLYSIVCGKSTPEKE 256
FAS IV---------WVKRKEV---QKTCRKHR 203
Consensus #1 . . . . G . . . . P . . . . . .
DDCR NPPPGTHLSPLDSAHTLLAPPDSSEKICTV 285
TNFR1 GELEGTTTKPLAPNPSFSPTPGFTPTLGFS 286
FAS KENQGSHESP---------------- 214

FIGURE 3



Consensus 'Consensus #1': When all match the residue of the Consensus show the residue of the Consensus, otherwise show '.'.

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

FIGURE 3 (CONT'D)

(A) LENGTH: 1783 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 198..1481

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CATGGGTGGG GGTGGGGGCG CTGCTGGATT CCTGCTCTGG TGGAGGGGAA ACTTGTGAGG      60

GGCTGGTAAG CGCCCCCTCC GAAGCCTGGT GTGTGCGCGG GGGGAAGGAA GTTAGTTTCC     120

TCTCCACCCA TGGGCACCCC TTCTGCCCGG GGCCTGGGAA GTGGGCTGCT CTGTGGGCAA     180

ATGCTGGGGC CTCTGAA ATG GAG GAG ACG CAG CAG GGA GAG GCC CCA CGT          230
                  Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg
                    1               5                  10

GGG CAG CTG CGC GGA GAG TCA GCA GCA CCT GTC CCC CAG GCG CTC CTC        278
Gly Gln Leu Arg Gly Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu
              15                  20                  25

CTG GTG CTG CTG GGG GCC CGG GCC CAG GGC GGC ACT CGT AGC CCC AGG        326
Leu Val Leu Leu Gly Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg
          30                  35                  40

TGT GAC TGT GCC GGT GAC TTC CAC AAG AAG ATT GGT CTG TTT TGT TGC        374
Cys Asp Cys Ala Gly Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys
      45                  50                  55

AGA GGC TGC CCA GCG GGG CAC TAC CTG AAG GCC CCT TGC ACG GAG CCC        422
Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
  60                  65                  70                  75

TGC GGC AAC TCC ACC TGC CTT GTG TGT CCC CAA GAC ACC TTC TTG GCC        470
Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
                  80                  85                  90

TGG GAG AAC CAC CAT AAT TCT GAA TGT GCC CGC TGC CAG GCC TGT GAT        518
Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
              95                  100                 105

GAG CAG GCC TCC CAG GTG GCG CTG GAG AAC TGT TCA GCA GTG GCC GAC        566
Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
          110                 115                 120
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```
100  ACC CGC TGT GGC TGT AAG CCA GGC TGG TTT GTG GAG TGC CAG GTC AGC       614
101  Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
102      125                 130                 135
103
104  CAA TGT GTC AGC AGT TCA CCC TTC TAC TGC CAA CCA TGC CTA GAC TGC       662
105  Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
106  140                 145                 150                 155
107
108  GGG GCC CTG CAC CGC CAC ACA CGG CTA CTC TGT TCC CGC AGA GAT ACT       710
109  Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
110                  160                 165                 170
111
112  GAC TGT GGG ACC TGC CTG CCT GGC TTC TAT GAA CAT GGC GAT GGC TGC       758
113  Asp Cys Gly Thr Cys Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys
114              175                 180                 185
115
116  GTG TCC TGC CCC ACG AGC ACC CTG GGG AGC TGT CCA GAG CGC TGT GCC       806
117  Val Ser Cys Pro Thr Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala
118          190                 195                 200
119
120  GCT GTC TGT GGC TGG AGG CAG ATG TTC TGG GTC CAG GTG CTC CTG GCT       854
121  Ala Val Cys Gly Trp Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala
122      205                 210                 215
123
124  GGC CTT GTG GTC CCC CTC CTG CTT GGG GCC ACC CTG ACC TAC ACA TAC       902
125  Gly Leu Val Val Pro Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr
126  220                 225                 230                 235
127
128  CGC CAC TGC TGG CCT CAC AAG CCC CTG GTT ACT GCA GAT GAA GCT GGG       950
129  Arg His Cys Trp Pro His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly
130                  240                 245                 250
131
132  ATG GAG GCT CTG ACC CCA CCA CCG GCC ACC CAT CTG TCA CCC TTG GAC       998
133  Met Glu Ala Leu Thr Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp
134              255                 260                 265
135
136  AGC GCC CAC ACC CTT CTA GCA CCT CCT GAC AGC AGT GAG AAG ATC TGC      1046
137  Ser Ala His Thr Leu Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys
138          270                 275                 280
139
140  ACC GTC CAG TTG GTG GGT AAC AGC TGG ACC CCT GGC TAC CCC GAG ACC      1094
141  Thr Val Gln Leu Val Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr
142      285                 290                 295
143
144  CAG GAG GCG CTC TGC CCG CAG GTG ACA TGG TCC TGG GAC CAG TTG CCC      1142
145  Gln Glu Ala Leu Cys Pro Gln Val Thr Trp Ser Trp Asp Gln Leu Pro
146  300                 305                 310                 315
147
148  AGC AGA GCT CTT GGC CCC GCT GCT GCG CCC ACA CTC TCG CCA GAG TCC      1190
149  Ser Arg Ala Leu Gly Pro Ala Ala Ala Pro Thr Leu Ser Pro Glu Ser
150                  320                 325                 330
151
152  CCA GCC GGC TCG CCA GCC ATG ATG CTG CAG CCG GGC CCG CAG CTC TAC      1238
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153  Pro Ala Gly Ser Pro Ala Met Met Leu Gln Pro Gly Pro Gln Leu Tyr
154                335                 340                 345
155
156  GAC GTG ATG GAC GCG GTC CCA GCG CGG CGC TGG AAG GAG TTC GTG CGC      1286
157  Asp Val Met Asp Ala Val Pro Ala Arg Arg Trp Lys Glu Phe Val Arg
158          350                 355                 360
159
160  ACG CTG GGG CTG CGC GAG GCA GAG ATC GAA GCC GTG GAG GTG GAG ATC      1334
161  Thr Leu Gly Leu Arg Glu Ala Glu Ile Glu Ala Val Glu Val Glu Ile
162      365                 370                 375
163
164  GGC CGC TTC CGA GAC CAG CAG TAC GAG ATG CTC AAG CGC TGG CGC CAG      1382
165  Gly Arg Phe Arg Asp Gln Gln Tyr Glu Met Leu Lys Arg Trp Arg Gln
166  380                 385                 390                 395
167
168  CAG CAG CCC GCG GGC CTC GGA GCC GTT TAC GCG GCC CTG GAG CGC ATG      1430
169  Gln Gln Pro Ala Gly Leu Gly Ala Val Tyr Ala Ala Leu Glu Arg Met
170                400                 405                 410
171
172  GGG CTG GAC GGC TGC GTG GAA GAC TTG CGC AGC CGC CTG CAG CGC GGC      1478
173  Gly Leu Asp Gly Cys Val Glu Asp Leu Arg Ser Arg Leu Gln Arg Gly
174              415                 420                 425
175
176  CCG TGACACGGCG CCCACTTGCC ACCTAGGCGC TCTGGTGGCC CTTGCAGAAG           1531
177  Pro
178
179
180  CCCTAAGTAC GGTTACTTAT GCGTGTAGAC ATTTTATGTC ACTTATTAAG CCGCTGGCAC    1591
181
182  GGCCCTGCGT AGCAGCACCA GCCGGCCCCA CCCCTGCTCG CCCCTATCGC TCCAGCCAAG    1651
183
184  GCGAAGAAGC ACGAACGAAT GTCGAGAGGG GGTGAAGACA TTTCTCAACT TCTCGGCCGG    1711
185
186  AGTTTGGCTG AGATCGCGGT ATTAAATCTG TGAAAGAAAA CAAAACAAAA CAAAAAAAAA    1771
187
188  AAAAAAAAAA AA                                                       1783
189
190
191  (2) INFORMATION FOR SEQ ID NO:2:
192
193         (i) SEQUENCE CHARACTERISTICS:
194               (A) LENGTH: 428 amino acids
195               (B) TYPE: amino acid
196               (D) TOPOLOGY: linear
197
198       (ii) MOLECULE TYPE: protein
199
200       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
201
202  Met Glu Glu Thr Gln Gln Gly Glu Ala Pro Arg Gly Gln Leu Arg Gly
203    1               5                  10                  15
204
205  Glu Ser Ala Ala Pro Val Pro Gln Ala Leu Leu Leu Val Leu Leu Gly
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206                 20                  25                  30
207
208   Ala Arg Ala Gln Gly Gly Thr Arg Ser Pro Arg Cys Asp Cys Ala Gly
209             35                  40                  45
210
211   Asp Phe His Lys Lys Ile Gly Leu Phe Cys Cys Arg Gly Cys Pro Ala
212         50                  55                  60
213
214   Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro Cys Gly Asn Ser Thr
215   65                  70                  75                  80
216
217   Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala Trp Glu Asn His His
218                   85                  90                  95
219
220   Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp Glu Gln Ala Ser Gln
221               100                 105                 110
222
223   Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp Thr Arg Cys Gly Cys
224           115                 120                 125
225
226   Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser Gln Cys Val Ser Ser
227       130                 135                 140
228
229   Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys Gly Ala Leu His Arg
230   145                 150                 155                 160
231
232   His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr Asp Cys Gly Thr Cys
233                   165                 170                 175
234
235   Leu Pro Gly Phe Tyr Glu His Gly Asp Gly Cys Val Ser Cys Pro Thr
236               180                 185                 190
237
238   Ser Thr Leu Gly Ser Cys Pro Glu Arg Cys Ala Ala Val Cys Gly Trp
239           195                 200                 205
240
241   Arg Gln Met Phe Trp Val Gln Val Leu Leu Ala Gly Leu Val Val Pro
242       210                 215                 220
243
244   Leu Leu Leu Gly Ala Thr Leu Thr Tyr Thr Tyr Arg His Cys Trp Pro
245   225                 230                 235                 240
246
247   His Lys Pro Leu Val Thr Ala Asp Glu Ala Gly Met Glu Ala Leu Thr
248                   245                 250                 255
249
250   Pro Pro Pro Ala Thr His Leu Ser Pro Leu Asp Ser Ala His Thr Leu
251               260                 265                 270
252
253   Leu Ala Pro Pro Asp Ser Ser Glu Lys Ile Cys Thr Val Gln Leu Val
254           275                 280                 285
255
256   Gly Asn Ser Trp Thr Pro Gly Tyr Pro Glu Thr Gln Glu Ala Leu Cys
257       290                 295                 300
258
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60/028711

Provisional Application For Patent Cover Sheet

Address to:
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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. § 1.53(b)(2).

Docket Number: 1488.0310001	Type a plus sign (+) inside this box ☞	+

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1-00 / 5-00

TITLE OF THE INVENTION (280 Characters Maximum)

Death Domain Containing Receptors

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ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification *Number of pages*: 92 ☐ Small Entity Statement
Including Sequence Listing and Claims
☒ Drawing(s) *Number of sheets*: 6 ☒ Other (specify) Diskette copy of Sequence Listing

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☒ No
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Respectfully submitted,

Signature: Kimberlin M. Toohey Date: Oct 17, 1996
Typed or Printed Name: Kimberlin M. Toohey Registration No. 35,391

Death Domain Containing Receptor

Abstract

The present invention relates to novel Death Domain Containing Receptor (DR3 and DR3-V1) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR3 and DR3-V1 proteins. DR3 and DR3-V1 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR3 and DR3-V1 activity.

DR3.APL